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MORPHOLOGICAL VARIANTS IN NEURODEVELOPMENTAL DISORDERS

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Morphological Variants in Neurodevelopmental Disorders
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To my husband, David, and my children, Malin and Thor

ABSTRACT

Neurodevelopmental disorders (NDDs), including autism spectrum disorder (ASD) and attention-deficit/hyperactivity disorder (ADHD) are diagnosed in a significant minority of children, adolescents, and adults worldwide. Physical examinations are recommended for children undergoing assessment of NDDs, including evaluation of the presence of morphological variants, which are altered physical features of the body such as low-set ears, curved finger digits, narrow lips, etc. Morphological variants have been shown to appear more frequently in children with some NDDs, such as ASD, and in children with genetic syndromes. Physical examinations for morphological variants may help identify children undergoing assessment for NDDs who would benefit from further screening or testing (i.e., genetic). However, these physical examinations are often subjective, time-consuming, and require a high level of expertise to perform.

The thesis uses a cohort of twins recruited in the Roots of Autism and ADHD Twin Study in Sweden (RATSS) who received detailed in-person clinical and automated morphological assessments, neuroimaging, and molecular genetic testing. The aims of the studies in this thesis are to identify characteristics of morphological variants that can support NDD screening and risk assessment, to test whether it is possible to obtain reliable morphological assessments using low-cost, automated technology, and to utilize the twin design to explore the potential genetic and environmental influences on the development of morphological variants. Overall, it was hypothesized that individuals with NDDs would have increased numbers of morphological variants compared to those with typical development.

Study I explored the presence of minor physical anomalies (MPAs) in twin pairs in RATSS who received in-person clinical assessments and compared MPAs found in twins with NDDs to those with typical development. This study found that individuals with ASD in particular had increased numbers of MPAs, as well as those with increasing scores on a measure for autistic traits and those with lower IQs. Monozygotic twins, regardless of NDD diagnosis, had similar numbers and types of MPAs.

Study II explored the relationship of the 2nd and 4th finger digit ratio (2D:4D) in individuals with diagnoses of various NDDs and found a small decrease in the ratio in individuals with NDDs overall, but not for any particular NDD. Additionally, the study confirmed the

results of previous studies on 2D:4D ratios by finding lower ratios in males compared with females.

Study III detailed the presence of morphological variants, as well as diagnostic and behavioral findings, in a twin pair with an identified genetic mutation (i.e., duplication on Chromosome 12) and NDD diagnoses of ASD and ADHD. The study compared phenotypic findings in this twin pair with other individuals worldwide identified with a similar size and location of duplication. The twin pair, along with the other individuals with the duplication, were reported to have learning difficulties, cognitive impairment, language and gross motor delays, and at least one NDD (i.e., intellectual disability, ADHD, ASD). The twin pair and other individuals identified were primarily males and had morphological variants present in head shape, forehead, eyes, vision, ears, nose, oral-facial region, and toe digits.

Finally, Study IV compared in-person clinical assessments of facial morphological variants (FMVs) with automated assessments using Face2Gene (F2G), and described findings from the automated assessments in a large sample of twins with either typical development or NDDs. The study also explored differences in FMVs by presence of NDD diagnosis. The study found high to nearly complete agreement between clinical and automated assessments of FMVs. However, FMVs were neither increased, nor distinguishable, between individuals with NDD diagnoses versus those with typical development.

The studies in this thesis point to the potential value of morphological assessment as part of the physical examination in individuals with NDDs, particularly for those who may have NDDs such as ASD, increased autistic traits, lower IQs, or genetic variants. In general, the studies in this thesis found increased numbers of morphological variants in individuals with NDDs and in some with genetic variations (i.e., 12q12 duplication), but significant differences in the number of variants in individuals diagnosed with NDDs versus typical development were not always identified. Although automated assessments are now available to detect morphological variants, they are limited to just certain body regions (i.e., face). In contrast, in-person clinical assessments allow for a full, head-to-toe examination and appear to be better at identifying individuals with morphological variants. In conclusion, identification of morphological variants may point to individuals with possible NDDs or underlying genetic alterations and have the potential to help determine individuals undergoing diagnosis for NDDs who would most benefit from further screening or testing, such as genetic testing.

SCIENTIFIC PAPERS IN THIS THESIS

I. Myers, L., Anderlid, B. M., Nordgren, A., Willfors, C., Kuja-Halkola, R., Tammimies, K., & Bölte, S. (2017). Minor physical anomalies in neurodevelopmental disorders: a twin study. *Child and Adolescent Psychiatry and Mental Health, 11*, 57. doi:10.1186/s13034-017-0195-y

II. Myers, L., Van't Westeinde, A., Kuja-Halkola, R., Tammimies, K., & Bölte, S. (2018). 2D:4D ratio in neurodevelopmental disorders: A twin study. *Journal of Autism and Developmental Disorders, 48*(9), 3244-3252. doi:10.1007/s10803-018-3588-8

III. Myers, L., Blyth, M., Morakhani, K., Hranilovic, D., Polesie, S., Isaksson, J., Nordgren, A., Bucan, M., Vincent, M., Bölte, S., Anderlid, B.M., & Tammimies, K. (2019). Variable neurodevelopmental and morphological phenotypes of carriers with 12q12 duplication. *Molecular Genetics & Genomic Medicine.* (Accepted).

IV. Myers, L., Anderlid, B.M., Nordgren, A., Lundin, K., Kuja-Halkola, R., Tammimies, K., & Bölte, S. (2019). Clinical versus automated assessments of morphological variants in twins with and without neurodevelopmental disorders. (Submitted).

CONTENTS

LIST OF ABBREVIATIONS

2D	Two-Dimensional
2D:4D	2nd finger digit to 4th finger digit ratio
3D	Three-Dimensional
ADI-R	Autism Diagnostic Interview-Revised
ADOS	Autism Diagnostic Observation Schedule
ADHD	Attention-Deficit/Hyperactivity Disorder
APA	American Psychiatric Association
ASD	Autism Spectrum Disorder
ASEBA	Child Behavior Checklist and Adult Behavior Checklist
AUC	Area Under the Curve
CATSS	Child and Adolescent Twin Study Sweden
CMA	Chromosomal Microarray
CNV	Copy Number Variant
DCQ	Dysmorphic Concern Questionnaire
DECIPHER	Database of Genomic Variation and Phenotype in Humans using Ensembl Resources
DIVA 2.0	Diagnostic Interview for ADHD in Adults
DNA	Deoxyribonucleic acid
DSM-5	Diagnostic and Statistical Manual of Mental Disorders, 5th Edition
DTI	Diffusion Tensor Imaging
DZ	Dizygotic
EIBI	Early Intensive Behavioral Intervention
F2G	Face2Gene
fMRI	Functional MRI
FMV	Facial Morphological Variant
GEE	Generalized Estimating Equation
HPO	Human Phenotype Ontology
ICD	International Classification of Diseases
ID	Intellectual Disability
IF	Incidental Brain Magnetic Resonance Imaging (MRI) Findings

IQ	Intelligence Quotient
K-SADS	Kiddie Schedule for Affective Disorders and Schizophrenia
M	Mean
Md	Median
MPA	Minor Physical Anomaly
MRI	Magnetic Resonance Imaging
MZ	Monozygotic
NICE	National Institute for Health and Care Excellence
NDD	Neurodevelopmental Disorder
OMIM	Online Mendelian Inheritance in Man
PPVT-4	Peabody Picture Vocabulary Test, Fourth Edition
RATSS	Roots of Autism and ADHD Twin Study in Sweden
ROC	Receiver Operating Characteristic
R_s	Spearman Correlation
SD	Standard Deviation
SE	Standard Error
SRS-2	Social Responsiveness Scale-2
TD	Typical Development or Typically Developing
US	United States
WAIS-IV	Wechsler Adult Intelligence Scale-IV
WES	Whole Exome Sequencing
WISC-IV	Wechsler Intelligence Scale for Children-IV

1 INTRODUCTION

1.1 NEURODEVELOPMENTAL DISORDERS (NDDS)

The umbrella concept of neurodevelopmental disorders (NDDs) includes various types of conditions that emerge in early childhood and cause mostly persistent impairment in cognitive, social, academic, and/or occupational functioning. NDDs include autism spectrum disorder (ASD), attention-deficit/hyperactivity disorder (ADHD), intellectual disability (ID), communication disorders, specific learning disorders, and motor disorders. There is substantial overlap among the various NDDs, along with a sex ratio that is skewed towards males with most of the disorders [American Psychiatric Association (APA), 2013]. Although no estimates exist regarding the prevalence of NDDs as an overarching diagnostic category, Boyle et al. (2011) examined developmental disabilities (including learning disabilities, ASD, ADHD, and other developmental delays) in a sample of children from the United States (US) ages three to 17 years and found a prevalence of nearly 14%. Earlier detection of NDDs is critical as it allows the opportunity for early intervention (Sand et al., 2005; Sices, Feudtner, McLaughlin, Drotar, & Williams, 2004). Early intervention has been shown to dampen the effects of a delay from NDDs on a child's development and enhance the child and family's well-being (Council on Children With Disabilities, 2006; Sand et al., 2005).

Despite efforts to identify NDDs early, multiple studies suggest that the time between parents first noticing concerns about their child's development to receiving a diagnosis of a NDD can be quite prolonged (Fridman, Banaschewski, Sikirica, Quintero, & Chen, 2017; Miodovnik, Harstad, Sideridis, & Huntington, 2015; Zuckerman, Lindly, & Sinche, 2015). Various behavioral screening measures exist to detect some NDDs like ASD early (Lord, Elsabbagh, Baird, & Veenstra-Vanderweele, 2018). However, these measures are not always consistently used by health care providers (Constantino & Charman, 2016; Radecki, Sand-Loud, O'Connor, Sharp, & Olson, 2011; Sand et al., 2005), often due to issues including lack of training on use of instruments, where to refer children (King et al., 2010; Morelli et al., 2014), or the instruments do not perform well in correctly identifying young children at risk (Haglund, Dahlgren, Gustafsson, Råstam, & Källen, 2017).

A variety of biomarkers are being explored as possible ways to detect NDDs earlier or place individuals with NDDs into subgroups, which may help determine individuals who

would benefit most from different types of assessments or treatment or also help identify unique genes or brain mechanisms that underlie these disorders. Some examples of biological samples being explored as biomarkers include blood, teeth, cerebral spinal fluid, stool, and variations in physical appearance, or what are known as morphological variants (Bölte et al., 2014; Goldani, Downs, Widjaja, Lawton, & Hendren, 2014; Ruggeri, Sarkans, Schumann, & Persico, 2014). For morphological variants in particular, the examination of these variants has evolved through time with newer automated methods now available that may have the potential to assess variants effectively and objectively in individuals with NDDs.

1.2 AUTISM SPECTRUM DISORDER (ASD)

ASD is a complex neurodevelopmental disorder behaviorally characterized by impairments in social communication and interactions and the presence of repetitive, stereotyped behaviors (APA, 2013). Recent studies have shown high heritability of ASD, with estimates between 56-95% (Bai et al., 2019; Colvert et al., 2015; Ronald & Hoekstra, 2011; Sandin et al., 2017; Tick, Bolton, Happe, Rutter, & Rijsdijk, 2016). A report from the Centers for Disease Control and Prevention estimates the prevalence of ASD in children in the United States to be one in 59 children, or around 1.6% (Baio et al., 2018). A recent study from Stockholm County in Sweden shows an ASD prevalence of about 1.4% in children (0-12 years) and 3.1% in adolescents (13-17 years) (Kosidou, Edwin, Magnusson, & Dalman, 2017). A large majority of individuals with ASD have comorbid conditions, including other neurodevelopmental issues such as ADHD or ID; medical issues such as seizure or sleep disorders; and psychiatric and psychological issues such as anxiety or depression (Lai, Lombardo, & Baron-Cohen, 2014; Leyfer et al., 2006; Lord et al., 2018; Simonoff et al., 2008).

ASD is typically diagnosed with data obtained from multiple informants (e.g., parents or other caregivers, teachers, etc.) based on criteria set forth by the Diagnostic and Statistical Manual of Mental Disorders (DSM-5; APA, 2013) and ICD-10 (World Health Organization, 1992) and includes the aforementioned impairments in social communication and interaction and restricted and repetitive behaviors, interests, or activities. These issues present in early childhood and affect everyday functioning (APA, 2013). Recommendations exist to screen for ASD in the primary care setting beginning as early as 18 months of age (Johnson, Myers, & American Academy of Pediatrics Council on Children With Disabilities, 2007; Zwaigenbaum et al., 2015) and it is generally thought ASD can be

diagnosed reliably in children as young as two years of age (Constantino & Charman, 2016; Lai et al., 2014; Tonge, Bull, Brereton, & Wilson, 2014), although a recent study suggested ASD may be reliably diagnosed in children as young as 14 months of age (Pierce et al., 2019). However, it is important to assess the validity of diagnostic instruments for young children, as was done in Zander, Sturm, and Bölte (2015), which recommends the combined use of the Autism Diagnostic Interview-Revised (ADI-R; Rutter, LeCouteur, & Lord, 2003) and the Autism Diagnostic Observation Schedule (ADOS; Lord et al., 2012) for young children suspected to have ASD. Even with the ability to screen for and potentially diagnose ASD early, research shows children often receive a diagnosis later in childhood (Zuckerman et al., 2015). Children who receive timely diagnosis of ASD have the opportunity to receive early intervention with the potential to result in improved long-term outcomes for the child and family (Zwaigenbaum et al., 2015). Although no cure exists for ASD, a variety of behavioral interventions and medications have been explored for the treatment of symptoms associated with the disorder. However, the recommended interventions or treatments vary by individual due to the heterogeneity of the disorder, and research in this area is ongoing (Lai et al., 2014; Sahin & Sur, 2015; Tonge et al., 2014).

1.3 ATTENTION-DEFICIT/HYPERACTIVITY DISORDER (ADHD)

ADHD is a NDD arising in early to middle childhood and is behaviorally characterized by inattention and hyperactivity/impulsivity that interfere with quality of life or functioning (APA, 2013). Heritability estimates for ADHD are around 70-80% (Brikell, Kuja-Halkola, & Larsson, 2015). It is estimated that about 5.3-7.2% of children exhibit ADHD worldwide (Polanczyk, de Lima, Horta, Biederman, & Rohde, 2007; Thomas, Sanders, Doust, Beller, & Glasziou, 2015) and 2.0% of children (0-12 years) and 7.7% of adolescents (13-17 years) in Stockholm County, Sweden (Kosidou et al., 2017). Co-morbidity is also high in ADHD, with other co-occurring disorders including ASD, communication disorders, ID, and behavioral problems like conduct disorder and oppositional defiant disorder (as reviewed in Thapar & Cooper, 2016). Studies point to poor outcomes for individuals with a diagnosis of ADHD (Thapar & Cooper, 2016). For example, a study examining males diagnosed during childhood with ADHD found worse educational, economical, occupational, and social outcomes in adulthood compared with males without a diagnosis (Klein et al., 2012).

ADHD diagnosis is based on the ICD-10 definition of a hyperkinetic disorder or on criteria set forth in the DSM-5, which includes the child having experienced issues with inattention and/or hyperactivity and impulsivity, along with the presence of these issues before the age

of 12 years and in multiple settings. Additionally, the disorder must interfere with functioning and the symptoms must not be the result of another disorder or psychotic issues. ADHD is diagnosed through multiple methods with the use of multiple informants (APA, 2013; National Institute for and Care Excellence, 2018). Treatment guidelines for ADHD include those developed by the National Institute for Health and Care Excellence (NICE, 2018). The treatment guidelines are based on the child's age and recommendations include behavioral interventions like parent-training programs and environmental modifications (e.g., change in lighting or noise levels, reducing distractions) to help decrease issues for individuals with ADHD. For children over the age of five years, the use of stimulant medication (e.g., methylphenidate as first line agent) may be considered. Cognitive behavioral therapy may also be recommended for children who experience some improvement with medication, but still have problematic symptoms (NICE, 2018).

1.4 OTHER NDD

Other NDD is a phrase used in the DSM-5 to include ID, communication disorders, specific learning disorders, and motor disorders (APA, 2013). Key features of these disorders are that they often arise in childhood and tend to persist into adulthood (Thapar, Cooper, & Rutter, 2017). Similar to ASD and ADHD, disorders in other NDD are believed to be highly heritable with a high level of overlap (Thapar et al., 2017). ID is thought to affect around one percent of individuals worldwide. It is diagnosed through clinical testing with behavioral measures and standardized intelligence testing (APA, 2013; Maulik, Mascarenhas, Mathers, Dua, & Saxena, 2011). Communication disorders include issues with both expressive and receptive language abilities, including speech sound disorder, childhood-onset fluency disorder, and social communication disorder. For these disorders, diagnosis is based on the presence of communication difficulties that have an onset in early childhood and are not attributable to other disorders or medical issues (APA, 2013). Learning disorders include challenges related to learning in the areas of reading, math, or written expression. The issues may first present in early childhood or become noticeable when the child is older and more academic demands are placed on the child (APA, 2013). It is estimated around 7% of children have learning disorders (Boyle et al., 2011). Motor disorders involve issues with coordination and movement, including vocal and motor tics. Diagnosis is based on issues with motor ability that begin in early childhood and are not attributable to other disorders or medical issues (APA, 2013).

1.5 ETIOLOGY OF NDDS

1.5.1 Genetics of NDDs

Research into the area of genetics of NDDs is ongoing, particularly in NDDs like ASD and ADHD. As previously noted, heritability estimates for both disorders are high (Bai et al., 2019; Brikell et al., 2015; Colvert et al., 2015; Ronald & Hoekstra, 2011; Sandin et al., 2017; Tick et al., 2016). Genetic testing with chromosomal microarray analysis (CMA) to detect copy number variants (CNVs) is recommended as a first-tier evaluation for all children undergoing diagnosis of ASD and ID (Moeschler, Shevell, & Committee On Genetics, 2014; Schaefer, Mendelsohn, & Professional Practice and Guidelines Committee, 2013). Genetic testing is also recommended for individuals with developmental disabilities broadly (Miller et al., 2010). Further genetic testing may be performed based on family history, findings on clinical exam that are concerning for a genetic disorder (e.g., morphological variants), or co-morbid medical conditions which may suggest the presence of a genetic disorder (Moeschler et al., 2014; Schaefer et al., 2013).

Rare genetic variants with clinical significance are estimated to be present in about 16-30% of children with the ASD (Buxbaum, 2009; Tammimies et al., 2015), including CNVs (Lai et al., 2014; Levy et al., 2011; Lord et al., 2018; Lyall et al., 2017; Vorstman et al., 2017; Woodbury-Smith & Scherer, 2018). It is less clear what percentage of children with ADHD have identifiable high-risk rare genetic variants; however, many common variants are thought to be at play in the development of ADHD (Faraone & Larsson, 2018; Thapar & Cooper, 2016). Although genetic liability appears to be high for both disorders, it is generally believed that most cases are due to complex interactions between an individual's genes and their environment (Hallmayer et al., 2011; Lai et al., 2014; Lord et al., 2018; Sahin & Sur, 2015). For example, De Rubeis and Buxbaum (2015) present a genotype-phenotype model for ASD demonstrating familial risk for the disorder (e.g., heritable common and rare genetic variants), combined with high-risk events (e.g., de novo mutations) and non-genetic factors (e.g., parental age), that may place an individual over the threshold and result in the clinical manifestation of NDDs like ASD.

A study by De Rubeis and Buxbaum (2015) notes that between 600-1200 different genes may be involved in ASD. As previously noted, CNVs include duplications and deletions in the genome and can result in either excess or loss of genetic material, respectively. The location of the CNV may affect either the genes produced and/or how they are expressed

(Merikangas, Corvin, & Gallagher, 2009; Velinov, 2019). CNVs can be either inherited/familial or de novo (i.e., new) and can provide great insight into the etiology of disease phenotypes. Chromosomal loci with CNVs are well-documented in ASD (e.g., deletions in 15q11-15q13, 16p11.2, and 22q11.2) and some loci have also been identified in ADHD (e.g., duplications at 15q13.3 and 16p13.1)(Fernandez & Scherer, 2017; Vorstman et al., 2017; Williams et al., 2012; Williams et al., 2010; Woodbury-Smith & Scherer, 2018). However, De Rubeis and Buxbaum (2015) note that the identified effect of the mutations spanning the different genes is highly variable for the resulting phenotype. For example, De Rubeis and Buxbaum discuss well-known CNVs (i.e., 22q11.2 and 16p11.2 deletions) in which individuals with these deletions typically have ASD, although there are cases with these deletions who also have typical development. Affected genes in the area of CNVs (e.g., *SHANK3, NLGN4X, NRXN1, MAPK3, CHD2, CHD8, ANK2*, etc.) have been studied in order to explore potential etiological origins of NDDs (Coe et al., 2019; De Rubeis & Buxbaum, 2015; Fernandez & Scherer, 2017; Velinov, 2019; Vorstman et al., 2017; Woodbury-Smith & Scherer, 2018) and new discoveries are likely to continue through research in this area.

1.5.2 Environmental Risks for NDDs

A variety of environmental factors have been explored in connection with the development of ASD, including preconception risk factors (e.g., parental age), prenatal risk factors (e.g., exposure to medications like valproate or selective serotonin reuptake inhibitors; toxic chemicals; maternal immune activation), perinatal and postnatal risks (e.g., birth complications, preterm birth), as well as protective factors (e.g., prenatal folic acid supplementation)(Bölte, Girdler, & Marschik, 2018; Lord et al., 2018; Lyall et al., 2017; Mandy & Lai, 2016). Possible environmental factors for ADHD include prematurity, low birth weight, maternal smoking, and exposure to environmental toxins (Gustafsson & Kallen, 2011; Thapar & Cooper, 2016). As previously noted, it is believed that NDDs result from the interactions between an individual's genes and environmental risk factors (De Rubeis & Buxbaum, 2015; Hallmayer et al., 2011; Lai et al., 2014; Lord et al., 2018; Sahin & Sur, 2015).

1.6 TWIN STUDIES

There are several types of twin study designs that are available for research, including the classic twin design, the co-twin control design, and the matched case-control design. In general, twin designs are based on the fact that monozygotic (MZ) twins share nearly 100% of their genomes, while dizygotic (DZ) twins share approximately 50% of their genomes.

This distinction allows researchers to compare outcomes or characteristics in MZ with DZ twins to explore the possible contribution of genes or environment in their research questions. For example, greater similarity on an outcome for MZ versus DZ twins may indicate strong genetic influence (Martin, Boomsma, & Machin, 1997; Posthuma & Polderman, 2013).

For twin studies, two assumptions are generally made: 1) equal environment and 2) random assortative mating. Equal environment refers to the assumption that if twins, regardless of zygosity, are raised in the same home, no difference exists in the effect of the environment on MZ versus DZ twin pairs. A violation of equal environments may result in issues regarding estimates of heritability. Assortative mating refers to the assumption that there is random selection of partners for the parents of these twins (Sahu & Prasuna, 2016; Willfors, Tammimies, & Bölte, 2017). A violation of the assortative mating assumption might mean parents mate with individuals more phenotypically similar to themselves and may lead to DZ twins, in particular, who share more than 50% of their genomes (Sahu & Prasuna, 2016; Willfors et al., 2017).

Twin studies have several strengths and limitations. A strength is that most twins share a similar prenatal environment and some level of similarity in their upbringing if reared together. Regardless of zygosity, twins are also the same age and generally have the same parents (although it is possible for DZ twins to have separate fathers). These strengths are in contrast to sibling studies with offspring who are different ages and subsequently have different prenatal and postnatal environments and are more likely than twins to have different fathers. Additionally, twin studies can save both time and cost as they are generally less challenging than traditional case-control studies where one needs to find a matched control. Limitations to twin studies, however, center on whether or not being a twin places an individual at an increased risk for certain outcomes, and if results from twin studies can be generalized to non-twin populations (Martin et al., 1997; Sahu & Prasuna, 2016).

1.6.1 Twin Studies in NDDs

Several twin studies have been conducted in the area of NDDs, primarily ASD or ADHD (Bailey et al., 1995; Freitag & Retz, 2010; Ghirardi et al., 2018; Monterrey et al., 2017; Posthuma & Polderman, 2013; Ronald, Simonoff, Kuntsi, Asherson, & Plomin, 2008; Tick et al., 2016) due to their unique ability to explore genetic and environmental factors

influencing phenomena of interest. However, twin studies focusing on exploring NDDs as an overarching diagnostic entity altogether are more limited (Bölte et al., 2014; Posthuma & Polderman, 2013). Although some previous studies have identified twinning as a risk factor for ASD in particular (Betancur, Leboyer, & Gillberg, 2002; Greenberg, Hodge, Sowinski, & Nicoll, 2001), more recent studies have not found similar conclusions when comparing the rate of ASD diagnosis or traits in twins with those of singletons or in non-twins sibling studies (Curran et al., 2011; Hallmayer et al., 2002).

The twin studies in the Roots of Autism and ADHD Twin Study Sweden (RATSS) are generally based on both the co-twin control design (also known as discordant MZ pair design or within-twin pair differences design) and the matched case-control design. In the co-twin control design, MZ twins discordant for an outcome of interest are especially informative as there may be something unique that occurred for one twin and not the other that could have contributed or resulted in the outcome. In the matched case-control design, twins are matched on a variety of factors (e.g., age, socio-economic environment, other shared environment, etc.) and although this design is similar to a typical case-control design with unrelated individuals, it is less time-consuming and costly (Sahu & Prasuna, 2016; Twin Research Australia, n.d.; Willfors et al., 2017).

1.7 PHYSICAL FEATURES AS A BIOMARKER OF NDDs

Best practice guidelines recommend children undergoing diagnosis of NDDs like ASD and ADHD should receive a comprehensive physical examination, which, at least for ASD, was explicitly recommended to include an assessment for morphological variants (Johnson et al., 2007, NICE, 2018). Although Leo Kanner's classic paper on children with autism (1943) described the children as "essentially normal" (p. 248) in physical appearance, he identified a few altered physical features and medical issues in the children (i.e., large heads, some motor problems, and a few other specific medical issues with one of the children). Since Kanner's original description of physical features in children with ASD, researchers in the area of NDDs have had interest in morphological variants.

For some disorders with a genetic basis, an individual may present with characteristic physical features that aid in early detection and diagnosis of the disorder. A classic example is the characteristic facial features that are present in nearly all individuals with Down syndrome such as epicanthal folds and upslanted palpebral fissures (Ostermaier, 2019;

Starbuck, Reeves, & Richtsmeier, 2011). Therefore, identification of physical features that vary in individuals with NDDs compared to those with typical development (TD) could serve as a tool for early detection of individuals at risk for NDDs (Ruggeri et al., 2014). Consequently, a handful of researchers have explored the use of morphology, or the study of physical features, as a noninvasive method of early detection, as well as subgrouping of individuals with NDDs, particularly in ASD and ADHD (Angkustsiri et al., 2011; Miles et al., 2008; Minahim & Rohde, 2015; Ozgen, Hellemann, de Jonge, Beemer, & van Engeland, 2013; Ozgen et al., 2011). These studies have primarily relied on the use of time-consuming and subjective physical examinations. Facial readers and other automated forms of assessment have become available in recent years that may help quickly and accurately assess morphological features in individual, although research in this area is emerging (Aldridge et al., 2011; Gilani et al., 2015; Lumaka et al., 2017; Obafemi-Ajayi et al., 2015; Tripi et al., 2019; Vorravanpreecha, Lertboonnum, Rodjanadit, Sriplienchan, & Rojnueangnit, 2018).

1.8 MORPHOLOGICAL VARIANTS

1.8.1 Definition

Morphological variant is the phrase used in this thesis to describe physical features that are variations on those found generally in a population. Examples of morphological variants include low-set ears, deeply-set eyes, highly arched eyebrows, narrow nasal tip, and triangular face. Morphological variants often are described as “physical anomalies”, “phenotypic abnormalities”, or “dysmorphology” in the research literature. Although these terms are still extensively used in clinical practice and the literature, some view them as having a negative connotation since many morphological variants are actually quite common in the general population (Kong, 2019; Merks et al., 2006). Therefore, these variants may not be as “abnormal” as these some of the terms suggest, but rather alterations within a normal range, distal from disease. Although this thesis will generally describe morphological variants, terms like “dysmorphology” and “physical anomalies” will also be used to describe findings from other studies as these terms are still prevalent today in the literature.

Perhaps the most informative work on describing and defining the types of morphological variants comes from Jon Aase (Aase, 1990), Bryan Hall (Hall, 1993), Merks, van Karnebeek, Caron, and Hennekam (2003), and Judith Miles (2000, 2008). Using these

classic references, an illustration depicting the delineation of morphological variants as described in this thesis is presented in Figure 1.

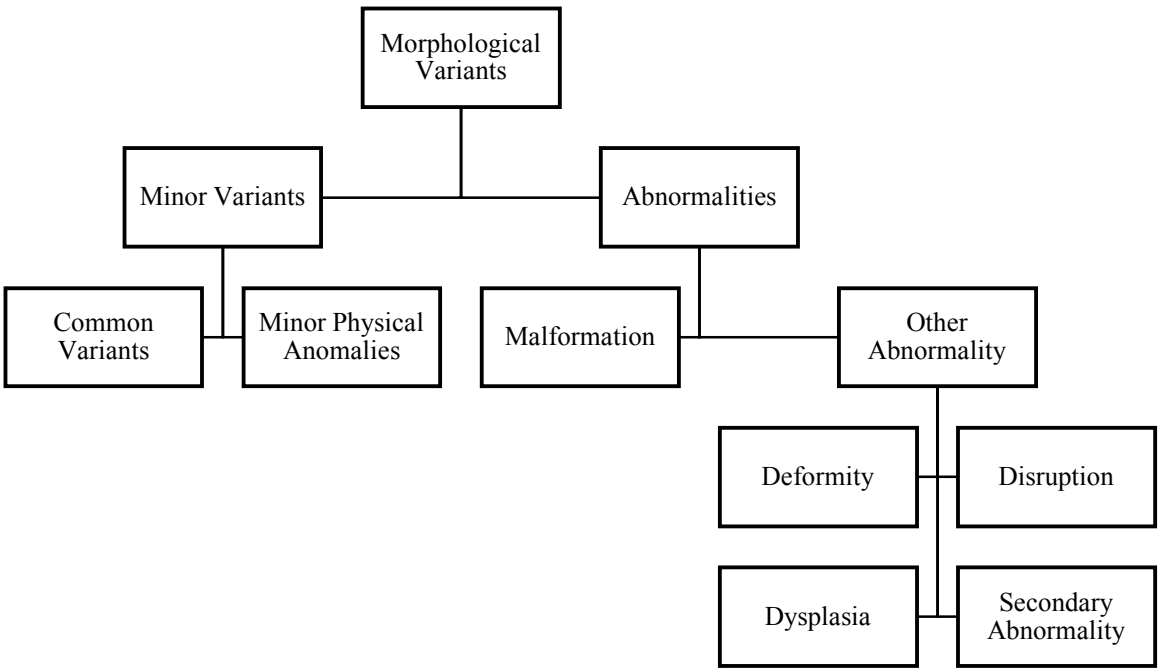

Figure 1. Delineation of morphological variants. Figure modified from Merks et al. (2003).

Morphological variants (what Merks and colleagues described as "phenotypic abnormalities") are divided into minor variants and abnormalities. Minor variants are further divided by factors related to both prevalence and implications of the variants, as was done in Merks et al. (2003) and include 1) common variants and 2) minor anomalies. Common variants are present in greater than 4% of the population, are considered normal variants, and include features like the curve of the fifth finger digit (i.e., clinodactyly), a gap between the first and second toes (i.e., sandal gap), sparse eyebrows, or a broad nose tip (Figure 2). Minor anomalies are generally now referenced in the literature as minor physical anomalies or MPAs (Miles, 2008). MPAs are rare and present in less than 4% of the population. They possibly indicate the presence of a major malformation or some type of disturbance in early development (Merks et al., 2003; Miles, 2008). MPAs are subtle physical features, like short palpebral fissures, a single, transverse palmar crease in the hand, and long toes (i.e., arachnodactyly)(Figure 2).

Figure 2. Images of minor variants and MPAs: clinodactyly (A); sandal gap (B); sparse eyebrows (C); broad nose tip (D); short palpebral fissures (E); single, transverse palmar crease (F); and long toes/arachnodactyly (G). Images courtesy of the National Human Genome Research Institute (genome.gov).

Abnormalities are another type of morphological variant and are defined to include either a 1) malformation (resulting from an issue during embryogenesis like a heart defect or facial cleft) or 2) other abnormality, like a deformity, disruption, dysplasia, or secondary abnormality. Hall (1993) and Merks et al. (2003) defined deformity as a normal fetal structure put into an abnormal position; disruption as a once normal structure altered more severely; dysplasia as an alteration in the development or functioning of a tissue; and secondary abnormality as an abnormality due to deficits in other structures. Of all the morphological variants in the area of NDDs, the variant that has received the most attention in research to date is MPAs, in part because of their potential association to either major malformations or disturbances in early development (Merks et al., 2003; Miles, 2008).

Miles and Hillman (2000) originally developed an often-cited classification system that indicates the level of dysmorphology based on total number of morphological variants, including 1) MPAs, 2) measurement abnormalities (e.g., macrocepahly, which indicates a head size two standard deviations from the mean), 3) descriptive traits (similar to common variants in that they occur in greater than 4% of the population, but can be also be familial), and 4) malformations. Miles and colleagues went on to suggest individuals with six or more MPAs or microcephaly be classified as dysmorphic, three to five MPAs as equivocal, and fewer than three MPAs as non-dysmorphic (Miles & Hillman, 2000; Miles et al., 2005; Miles et al., 2008). Miles et al. (2005) extended the original classification of dysmorphism to characterize individuals with six or more morphological variants as having “complex autism”, which Miles et al. argued provides evidence of abnormal embryological development. Miles et al. also suggested that this group with “complex autism” may represent individuals with a genetic syndrome resulting in ASD. Individuals with what Miles et al. termed “essential autism” had either few, if any morphological variants, or lacked microcephaly, which resulted in them being classified as “non-dysmorphic.” Miles et al. thought individuals with “essential autism” had a more heritable cause to ASD, rather than a genetic syndrome like those with complex autism. Miles et al. suggested that this

separation of the child with ASD into "complex" and "essential" groups might help with determining the child's prognosis and aid in counseling the family, especially if a genetic etiology to ASD is suspected.

Morphological variants are named according to the Human Phenotype Ontology (HPO), which includes standardized terms for nearly 13,000 morphological variants (Köhler et al., 2019). These terms are derived from the medical literature, along with genetic and rare disease resources (e.g., OMIM, DECIPHER, and Orphanet), and each term is used to identify a particular phenotypic feature (Köhler et al., 2019). HPO terms can be general (e.g., abnormality of the nasal tip) or very specific (e.g., narrow nasal tip) and each have a unique identification number (e.g., abnormality of the nasal tip=436; narrow nasal tip=11832).

1.8.2 Etiology of Morphological Variants

During early embryogenesis, the brain and skin are derived from the same neuroectodermal layer; therefore, morphological variants may mirror abnormal brain development since both systems are developing simultaneously and molecular signals determining the growth and development of one system may affect the other (Jones, 2013; Marcucio, Hallgrimsson, & Young, 2015). It is thought that morphological variants, and MPAs in particular, could suggest the possibility of an underlying genetic and/or environmental perturbation that affected early embryogenesis and led to both the variants and abnormal brain development, resulting in NDDs like ASD and ADHD (Accardo, Tomazic, Morrow, Haake, & Whitman, 1991; Angkustsiri et al., 2011; Dawson, Glasson, Dixon, & Bower, 2009; Gualtieri, Adams, Shen, & Loiselle, 1982; Jones, 2013; Manouilenko, Eriksson, Humble, & Bejerot, 2014; Miller et al., 2005; Minahim & Rohde, 2015; Ozgen, Hop, Hox, Beemer, & van Engeland, 2010; Ploeger, Raijmakers, van der Maas, & Galis, 2010; Timonen-Soivio et al., 2015).

Classic studies on morphological variants have shown that the presence of multiple MPAs may place an individual at an increased risk of a major malformation (Leppig, Werler, Cann, Cook, & Holmes, 1987; Marden, Smith, & McDonald, 1964), which is defined as a defect that has "surgical, medical, or cosmetic importance" (Leppig et al., 1987, p. 531). Additionally, past research has shown morphological variants like MPAs are stable over time (Quinn, Renfield, Burg, & Rapoport, 1977), although some variants can be acquired postnatally due to some type of acute or chronic event, including birth trauma or metabolic disorders, both of which may result in acquired morphological variants (Hall, 1993).

Additionally, in terms of the long-term implications of morphological variants, one study was identified that positively correlated the presence of MPAs identified during childhood with diagnoses of psychiatric disorders (e.g., anxiety and depression) and symptoms of paranoia in adulthood (Cheng, Chang, Chang, Lee, & Tzang, 2014).

Little is known about the heritability of morphological variants (Compton, Chan, Walker, & Buckley, 2011), although some studies have demonstrated higher amounts of morphological variants in siblings of individuals affected with neurodevelopmental or neuropsychiatric disorders compared with unrelated, TD controls (Dawson et al., 2009; Ismail, Cantor-Graae, & McNeil, 2000). However, a classic study of Canadian children with diagnoses of ASD or developmental delays and their TD siblings, along with matched TD controls, found mostly no difference in the types of MPAs in the sibling groups compared with controls (Rodier, Bryson, & Welch, 1997), but several significant differences in MPAs between those diagnosed with ASD or developmental delay and their siblings or controls.

Studies have also explored the relationship between MPAs and prenatal or perinatal complications. Quinn and Rapoport (1974) found increased counts of MPAs in children of mothers who reported issues like bleeding or toxemia (i.e., pre-eclampsia) during pregnancy, as well as babies born premature, small for gestational age, or through Cesarean section. In later studies (Firestone & Prabhu, 1983; Links, Stockwell, Abichandani, & Simeon, 1980) and a review (Firestone & Peters, 1983), researchers concluded that there was a relationship between increased MPAs and prenatal and perinatal complications. In a follow-up study by Links (1980), increased maternal age was also correlated with higher MPA scores, citing the potential for more genetic alterations occurring in offspring of older mothers (e.g., child with Down Syndrome). Finally, a few studies have suggested potential timing for the development of morphological variants during pregnancy, with some suggesting their development during the first trimester (Links et al., 1980; Timonen-Soivio et al., 2015), while another suggests timing after the first trimester (Tripi et al., 2008).

1.8.3 Prevalence of Morphological Variants

Morphological variants, especially MPAs, are common and can be present in individuals without any accompanying diagnosis or disorder. A classic study by Marden et al. (1964) found a prevalence of at least one MPA in about 14% of the study's healthy infant sample. Later studies also explored rates of variants in TD samples. Ulovec et al. (2004) found an

average of 1.70 variants (range of 0-5) in nearly 250 TD European school children using an 18-item dysmorphology scale (i.e., Waldrop). Merks et al. (2006) explored morphological variants in a sample of school-age children (8-14 years) recruited from a specific geographical region in the Netherlands (i.e., Kennemerland region) in mid-sized city and smaller, surrounding towns. The study included a clinical assessment performed by a physician trained in morphology and a clinical geneticist on a sample of nearly 1000 school children. Merks et al. identified an extensive list of head to toe morphological variants in the school-aged children ranging in prevalence from 0% (e.g., facial cleft, coloboma, protruding tongue, etc.) to just over 25% (e.g., sandal gap). Subsequent studies conducted in the Netherlands by Ozgen and colleagues examined 683 morphological variants in children with ASD and in TD controls and found that in one study (2013), more than 200 controls had an average of 9.5 morphological variants (range 2-26), 0.3 major abnormalities (range 0-2), 5.7 MPAs (range 2-13), and 3.2 common variants (range 0-10). In a previous study by Ozgen and colleagues (2011), nearly 60% of controls had at least one or more MPAs, while 25% had two or more MPAs. The areas with the highest frequency of MPAs for the controls in the Ozgen et al. (2011) study were joints (hypermobility in 9.3%), mouth (high palate in 7.6%), and the eyes (deep-set in 4%).

1.8.4 Measurement/Assessment of Morphological Variants

In many of the studies on morphological variants to date, including some classic studies, (Accardo et al., 1991; Bailey et al., 1995; Gualtieri et al., 1982; Manouilenko et al., 2014; Minahim & Rohde, 2015; Tripi et al., 2008; Ulovec et al., 2004; Waldrop & Goering, 1971), morphological variants, primarily MPAs, were assessed through either the original or modified dysmorphology scale known as the Waldrop Scale (Waldrop, Pedersen, & Bell, 1968). The scale was reported to be first developed by Goldfarb and Botstein (1956). These researchers used the scale in a study to evaluate physical features in children with schizophrenia, though the scale actually included MPAs noted in children with Down syndrome (Waldrop et al., 1968). Scores on the original scale ranged from 0-18 (or 0-30 if weighted). Items on the original scale include assessments for hair whorls, epicanthus, hypertelorism, adherent ear lobes, low seated, malformed, asymmetrical, soft and pliable ears, high palate, fifth finger curvature, single transverse palmar crease, longer index finger than middle finger, longer third toe compared with second toe, partial syndactylia of the two middle toes, gap between the first and second toe, and what was originally described at the time as electric hair (Waldrop et al., 1968). In a later study using the scale (Accardo et al., 1991), the researchers determined individuals with MPA scores less than three to five

on the Waldrop scale were considered to fall in the category classified as normal in morphological appearance, while those with scores greater than five were abnormal in morphological appearance.

A more recently developed scale, the Autism Dysmorphology Measure (Miles et al., 2008), has been used in only a handful of other studies to date (e.g., Angkustsiri et al., 2011; Flor, Bellando, Lopez, & Shui, 2017; Spencer, Takahashi, Chakraborty, Miles, & Shyu, 2018; Zachariah, Oommen, Padankatti, Grace, & Glory, 2017). In contrast to the Waldrop Scale, the Autism Dysmorphology Measure was specifically developed from variants found on physical examinations of children with ASD (Miles et al., 2008). The scale consists of 12 body areas that are evaluated to arrive at a designation of either dysmorphic or non-dysmorphic. The ADM demonstrates good reliability and validity and the measure is reported to be appropriate for use by health care providers with little training in dysmorphology (Miles et al., 2008).

Other studies have used a combination of scales (including the Waldrop) and/or checklists developed by the study researchers without previous validation to examine morphological variants in order to create an overall score based on the count of total variants present for analysis (Miles & Hillman, 2000; Ozgen et al., 2011; Rodier et al., 1997; Tammimies et al., 2015; Tripi et al., 2008; Wong, Fung, & Wong, 2014). Some researchers have also used Magnetic Resonance Imaging (MRI) scans to measure things such as interorbital or interlens distances on faces of participants (Cheung et al., 2011; Hardan, Keshavan, Sreedhar, Vemulapalli, & Minshew, 2006), citing the use of this imaging technology to provide precise measurements and to reduce examiner bias as the participant is generally not seen in person by the researcher conducting the measurements of the features.

Automated morphology assessment is a relatively new method to aid in the identification of morphological variants and genetic disorders. One of the early studies on the use of an automated morphology analysis systems was published by Aldridge et al. (2011) and involved a digital system (i.e., 3dMD Cranial System) to acquire three-dimensional (3D) facial images and measurements of the faces from boys diagnosed with ASD and TD controls. The study found differences in the facial features of the boys diagnosed with ASD compared with controls and was able to identify subgroups of boys with ASD based on facial dysmorphology who had distinct clinical and behavioral findings. The study concluded that the use facial dysmorphology as a biomarker for early identification and

subgrouping of ASD was promising. A later study by Obafemi-Ajayi et al. (2015) used similar methods to Aldridge et al. and found facial features could be used to separate boys with ASD into meaningful subgroups with differing clinical and behavioral profiles.

More recent articles have been published on the use of the Facial Dysmorphology Novel Analysis system (FDNA; Boston, MA.) or the technology behind it called DeepGestalt (Gurovich et al., 2017; Gurovich et al., 2019). DeepGestalt uses facial recognition software to analyze simple two-dimensional (2D) images to detect potential dysmorphic features and the relationship of these features to genetic syndromes (Gripp, Baker, Telegrafi, & Monaghan, 2016; Gurovich et al., 2017; Gurovich et al., 2019). To use the DeepGestalt system, a user submits a photo through the web platform of Face2Gene (F2G), which is freely available to medical professionals and researchers. The system first detects the face and then 130 facial points. From this, the system is able to measure areas on the face to help detect dysmorphic features along with comparing the facial image with a gestalt associated with syndromes the system is trained to detect (Basel-Vanagaite et al., 2016; Gurovich et al., 2017; Gurovich et al., 2019). When using F2G, users receive a list of potential genetic syndromes that are most closely related to the gestalt image produced through the use of the system and also a heat map of the participant's facial image, demonstrating areas in red that are most similar to those of others with the listed potential genetic syndromes (Basel-Vanagaite et al., 2016; Gurovich et al., 2017; Gurovich et al., 2019; Lumaka et al., 2017).

Recent studies have demonstrated F2G to be as good as or superior to clinical assessments performed by trained health care providers in identifying individuals with a variety of genetic conditions (i.e., mutations in the BAF complex genes in Gripp et al., 2016; XLHED phenotype in Hadj-Rabia et al., 2016; Cornelia de Lange in Basel-Vanagaite, 2016; Emmanuel and Pallister-Killian Syndromes in Liehr et al., 2017; Fetal Alcohol Syndrome and Alcohol-Related Neurodevelopmental Disorder in Valentine et al., 2017; inborn errors of metabolism in Pantel et al., 2018; and Down syndrome in Vorravanpreecha, Lertboonnum, Rodjanadit, Sriplienchan, & Rojnueangnit, 2018). Gurovich et al. (2017) specifically report that F2G has a 91% accuracy rate in identifying the correct disorder within the top 10 syndromes it lists for individuals for over 215 genetic syndromes. Gurovich et al. also note the system has been trained on a publicly available dataset of nearly 500,000 images from nearly 11,000 individuals and then refined on a proprietary database of images of participants with over 2500 genetic syndromes from F2G. One of the primary advantages of F2G is that it does not require the expensive and sophisticated

equipment needed to take 3D images like what was used in the study by Aldridge et al. (2011). Instead, facial photographs taken with a standard camera or phone can be used (Basel-Vanagaite et al., 2016; Gurovich et al., 2017). Additionally, F2G has the potential to be used by medical professionals and researchers who have limited access to trained dysmorphologists or clinical geneticists to be able to screen patients or participants, respectively, for genetic syndromes (Hadj-Rabia et al., 2017).

1.9 MORPHOLOGICAL VARIANTS IN NDDs

1.9.1 Morphological Variants in ASD

Evidence has demonstrated the presence of morphological variants in approximately 10-20% of the individuals with ASD (Angkustsiri et al., 2011; Miles & Hillman, 2000; Miles et al., 2005; Wong et al., 2014). Many of these studies have attempted to explore if individuals with ASD have particular morphological variants. Some of these studies report morphological variants that are more common in individuals with ASD, while others report that the overall number of morphological variants, rather than specific variants, is elevated in individuals with ASD.

1.9.1.1 Specific Morphological Variants in ASD

Tripi et al. (2008) specifically found abnormal head circumference (i.e., macrocephaly), abnormal cephalic index (i.e., dolichocephalic), and abnormal palates (i.e., high steepled) were more common in children diagnosed with ASD compared with TD controls. In the publication describing validation of the Autism Dysmorphology Measure, Miles et al. (2008) reported that in 25 of the 34 body regions assessed in their study were statistically significantly different ($p<.05$) between dysmorphic and non-dysmorphic participants with ASD. These regions included fingers/thumbs, oral cavity, nails, nose structure, face, philtrum, eyelids/palpebral fissures, mouth and lips, hair growth pattern, cranial shape, eyebrows, nose size, genitalia, stature, ear structure, feet, hands, forehead, abdomen, eye placement, back and spine, upper limbs, thorax and shoulders, teeth, and neck. Wong et al. (2014) conducted a study on over 1200 children with ASD exploring a variety of morphological variants and found macrocephaly, prominent forehead, big ears, and hypertelorism as the most commonly occurring morphological variants.

Studies have also examined the association between birth defects and/or congenital anomalies with a diagnosis of ASD and have found that children with ASD are more likely to have congenital anomalies (Schendel, Autry, Wines, & Moore, 2009; Timonen-Soivio et

al., 2015; Wier, Yoshida, Odouli, Grether, & Croen, 2006). One of the studies explored the presence of congenital anomalies, including some MPAs, specifically in a Nordic population (i.e., Finnish) of children with ASD (Timonen-Soivio et al., 2015). The study looked at the association between anomalies that were identifiable through a national database and the presence of a diagnosis of ASD and found several anomalies that were more likely to be present in individuals with ASD compared with matched controls. These anomalies included hypertelorism, hydrocephalus, dysmorphic facial features, high palate, macrocephaly, coloboma, and cataracts. In the same study, children with concurrent ID and ASD were even more likely to have anomalies than those with ASD alone in several body systems (i.e., eye, face/neck, cardiovascular, central nervous system, gastrointestinal, genitourinary, and musculoskeletal).

Some studies have also focused only on the facial region in the assessment of morphological variants due to the connection between the brain and face developing simultaneously in utero (Aldridge et al., 2011; Hammond et al., 2008; Obafemi-Ajayi et al., 2015). A recent commentary reviewed the facial findings in studies to date in participants with ASD and described some similar facial morphological variants, notably facial asymmetry, along with reported facial masculinity in both boys and girls with ASD compared with TD controls (Boutrus et al., 2017). Similarly, other recent studies have identified hypermasculinized facial features in both boys and girls with ASD compared with controls and less feminine facial features in females with ASD (Bejerot et al., 2018; Tan et al., 2017).

1.9.1.2 Overall Number of Morphological Variants in ASD

Although various studies have explored the presence of specific morphological variants in children with ASD, they have failed to produce a consistent list of variants identified across studies. Therefore, it may be more useful to examine the number of morphological variants as a way to determine if some type of embryological or fetal insult or genetic aberration may have occurred during development that contributed to not only the variant, but potentially also to a diagnosis of ASD. In fact, Ozgen and colleagues (2010) conducted a meta-analysis comparing effect sizes among seven studies exploring the number of MPAs specifically and found significantly higher numbers of MPAs for participants with ASD compared with TD controls, supporting the clinical assessment for the number of MPAs in particular as a potential biomarker for ASD. Later, Ozgen et al. (2013) utilized a checklist of 683 major and minor anomalies and found individuals with ASD had on average, 10.6

MPAs (range 4-22), which was significantly different compared with individuals in the control group who had only 5.7 MPAs (range 2-13). Subsequently, the number of MPAs, rather than specific MPAs, may be helpful in distinguishing individuals with ASD from other NDDs or TD.

One study to date has examined dysmorphology in twins in relation to a diagnosis of ASD, but the assessment was based only on a small number of items that were part of the previously mentioned Waldrop scale and the analyses and subsequent results were only minimally described (Bailey et al., 1995). The study did show that the proband most often had a higher number of MPAs compared with their co-twin, in twin pairs who were discordant for a diagnosis of ASD.

A handful of studies have used the presence of morphological variants to categorize children with ASD into subgroups with distinct clinical and/or behavioral phenotypes, including severity of ASD (Aldridge et al., 2011; Miles & Hillman, 2000; Miles et al., 2005; Obafemi-Ajayi et al., 2015; Wong et al., 2014). An additional study correlated the presence of dysmorphology with treatment outcomes in children receiving early intensive behavioral intervention (EIBI) in ASD (Stoelb et al., 2004). The study found that children lacking dysmorphic features were most likely to have better scores on a performance scale measuring attainment of EIBI treatment milestones at both six and 12 months post-treatment. The ability to use the clinical assessment for morphological variants as a possible way to help classify individuals with NDDs into subgroups could have implications for future diagnosis, treatment, or prediction of outcomes.

1.9.1.3 Finger Digit Ratio in ASD

The ratio comparing the length of the 2nd finger digit to the 4th finger digit, often referred to as the 2D:4D ratio, is another type of physical feature that has been explored quite extensively in the field of ASD. Typically, in males, the second digit is shorter than the fourth digit. In comparison, females generally have a more equal length of the second and fourth digits (Zheng & Cohn, 2011). The differences in these digit lengths is believed to be due to fetal hormone exposure, with higher levels of testosterone resulting in greater differences in length between the second and fourth digits (Galis, Ten Broek, Van Dongen, & Wijnaendts, 2010; Hampson, Ellis, & Tenk, 2008; Lutchmaya, Baron-Cohen, Raggatt, Knickmeyer, & Manning, 2004; Malas, Dogan, Evcil, & Desdicioglu, 2006; Manning,

Bundred, & Flanagan, 2002; Manning, Scutt, Wilson, & Lewis-Jones, 1998; Manning, Stewart, Bundred, & Trivers, 2004; Voracek & Dressler, 2007).

Research on the digit ratio in NDDs has primarily been done with ASD, in part, due to the extreme male brain theory (Baron-Cohen, Knickmeyer, & Belmonte, 2005), which suggests the influence of testosterone on the development of ASD. Evidence on the association between ASD and the ratio is inconsistent. Some studies have found lower 2D:4D ratios associated with ASD (Al-Zaid, Alhader, & Al-Ayadhi, 2015; de Bruin, de Nijs, Verheij, Verhagen, & Ferdinand, 2009; Honekopp, 2012b; Manning, Baron-Cohen, Wheelwright, & Sanders, 2001). Conversely, a recent, large study found no relationship between ASD diagnosis and the ratio (Guyatt, Heron, Knight Ble, Golding, & Rai, 2015). In one of the rare studies on the digit ratio in individuals with a variety of NDDs, as well as psychiatric disorders, de Bruin et al. (2009) identified lower 2D:4D ratios in males with ASD or ADHD compared with those with anxiety disorders or TD. The ratio has been studied once previously in twins, but only those with TD (Voracek & Dressler, 2007). The main finding of the study was that the ratio was highly heritable (with estimates around 80% for genetic effects).

1.9.2 Morphological Variants in ADHD

Older studies have primarily examined the relationship between hyperactivity and/or attention deficit disorder with MPAs in children and have demonstrated mixed results. Pomeroy, Sprafkin, and Gadow (1988) and Accardo et al. (1991) found no association between MPAs and the presence of hyperactivity or attention deficit disorder, while Waldrop et al. (1968), Quinn and Rapoport (1974), and Gualtieri et al. (1982) found significantly higher numbers of MPAs in children diagnosed with attention deficit disorder, hyperactivity, or ADHD. A study by Waldrop and Goering (1971) also found a relationship between an increased number of MPAs and presence of behavioral variables related to hyperactivity in an older sample of school age boys, but not for girls. A more recent study by Minahim and Rohde (2015) examined adults diagnosed with ADHD and found a significant association with the diagnosis and an increasing number of MPAs. Examples of MPAs found by Minahim and Rohde included hair whorls, high palate, clinodactyly, gap between first and second toes, and a thin upper lip. As evidence regarding the presence of morphological variants in individuals with ADHD is more limited, further studies are needed to explore both the type and number of morphological variants in individuals with ADHD and whether or not they may serve as a biomarker for ADHD.

1.9.3 Morphological Variants in other NDDs

Morphological variants have been only minimally studied in other NDDs beyond ASD and ADHD. An older study by Pomeroy et al. (1988) used a standardized checklist to determine developmental delay, including factors related to language and motor delays and learning difficulties. The study found a significant relationship between higher amounts of MPAs and the presence of developmental delay. In studies by Accardo et al. (1991) and Links et al. (1980), the researchers explored the relationship between MPAs and ID and found a significant association between scores on a dysmorphology measure (i.e., modified Waldrop scale) and intelligence quotient (IQ). However, Accardo et al. found that as IQ increased, the score on the dysmorphology measure also increased, whereas Links et al. found that children with ASD with higher numbers of MPAs were more likely to have lower IQs. A more recent study by Ulovec and colleagues (2004) found significant differences in the number of MPAs in children with developmental delays (including ID and visual and auditory delays) compared with children without delays. Children with delays had significantly higher numbers of MPAs (average of 3.61 MPAs in children with developmental delays compared with 1.70 MPAs in TD children). Angkustsiri et al. (2011) explored MPAs in children with developmental delay (defined as low cognitive and adaptive function) compared with those with ASD and TD and found significantly higher ratings of dysmorphology (3 or more MPAs) in children with developmental delay (nearly 50%), compared with ASD (17.4%) and TD (5.4%). As previously mentioned, de Bruin et al. (2009) examined 2D:4D finger digit ratios in individuals with a variety of NDDs and psychiatric disorders and found lower ratios in individuals with both ASD and ADHD compared with individuals with anxiety or TD controls.

1.9.4 Morphological Variants and Genetics

Morphological variants have been explored by researchers in the area of NDDs primarily because they may suggest underlying genetic and/or environmental perturbations that affected early embryogenesis, resulting in both the variant and neurodevelopmental disorders like NDDs (Accardo et al., 1991; Angkustsiri et al., 2011; Dawson et al., 2009; Gualtieri et al., 1982; Jones, 2013; Manouilenko et al., 2014; Miller et al., 2005; Minahim & Rohde, 2015; Ozgen et al., 2010; Ploeger et al., 2010). Genetic testing is recommended for all children undergoing diagnosis of ASD and further testing may be recommended in the event that the clinical assessment, review of family history, and/or co-morbid conditions indicate a possible underlying genetic condition (Schaefer et al., 2013). Genetic testing is also recommended in children with developmental disabilities and children with multiple

congenital anomalies (Miller et al., 2010). As a result, the assessment for morphological variants is one way health care providers may determine children and adolescents who would benefit from further genetic testing. Despite recommendations for such assessments as part of the diagnosis of NDDs such as ASD and ADHD (Johnson et al., 2007, NICE, 2018, Robert et al., 2017), little is known how often these occur and/or the training and level of expertise of health care providers needed to be able to perform these often subjective and complicated assessments.

Several studies have demonstrated a relationship between morphological variants, including MPAs and congenital anomalies, and the presence of genetic aberrations like CNVs (Engels et al., 2007; Girirajan et al., 2011; Miles & Hillman, 2000; Miles et al., 2005; Tammimies et al., 2015). Miles and colleagues (2000; 2005) demonstrated higher percentages of individuals with dysmorphology who had detectable genetic syndromes. Engels et al. (2007) found abnormal facial features in the study participants with ID who also had CNVs and concluded that the severity of the phenotype of the participants was based on the number of genes in the region of the CNV rather than the size. In the study by Girirajan et al. (2011), individuals with ID and multiple congenital anomalies had increased CNV burdens compared to those with just ID alone. Tammimies et al. (2015) found that children with increasing morphological variants were more likely to have higher diagnostic yields through genetic testing, especially using combinations of genetic testing methods like CMA and whole exome sequencing (WES). These studies together support the importance of the physical examination for morphological variants in the diagnosis of NDDs like ASD in help determine children who would most benefit from next steps like genetic testing to uncover the potential etiology of the disorders.

1.9.5 Morphological Variants and Neuroimaging

It has been said that the "face predicts the brain" (Demyer, Zeman, & Palmer, 1964), as the development of both the face and brain occur simultaneously in utero and the neural crest cells developing the facial region are derived from the dorsal neural tube, which eventually is associated with sensory functions in the nervous system (Marcucio et al., 2015). Because of these connections, along with molecular signals guiding the growth and development of these two body systems, the development of the face is highly dependent on the development of the brain (Marcucio et al., 2015) and morphological variants in the facial region may mirror abnormal brain development (Cheung et al., 2011; Demyer et al., 1964).

Although MRI scans are not routinely recommended in the assessment of children with NDDs such as ASD (Filipek et al., 2000), some studies have specifically explored structural brain abnormalities in individuals with morphological variants such as MPAs (Miles & Hillman, 2000; Miles et al., 2005; Wong et al., 2014) due to the connection between the development of the brain and the face (Cheung et al., 2011; Jones, 2013). Miles and Hillman (2000), Miles et al. (2005), and Wong et al. (2014) all found children with ASD and increased dysmorphic features were significantly more likely to have abnormal MRI scans. For example, Miles and Hillman (2000) identified structural alterations affecting the frontal, temporal, parietal, and cerebellar lobes, along with the corpus callosum in individuals with MPAs using MRIs. These studies suggested the possibility of using information related to both morphological variants and abnormal brain MRIs to classify individuals into subgroups in order to help determine etiology and prognosis for individuals with ASD, along with the future potential for tailored treatment options that may vary based on subgroup classification.

1.9.6 Limitations of Previous Research on Morphological Variants in NDDs

There are multiple limitations in studies to date on morphological variants in NDDs, including limitations in samples, measures and assessments, and methods.

1.9.6.1 Samples

Very little evidence has shown to what degree a relationship exists between morphological variants and the presence of NDDs broadly, especially with studies using well-controlled genetic backgrounds. Studies exploring morphological variants in individuals with NDDs as an overarching diagnostic entity, versus just ASD or ADHD alone, could be valuable, especially considering the substantial overlap among NDD diagnoses. In fact, Ozgen et al. (2013) discussed the need to examine variants in individuals with other NDDs beyond ASD, including ADHD, as well as other psychiatric disorders in their study exploring the predictive power of morphological variants in distinguishing individuals with ASD from those with TD. Additionally, studies exploring the presence of morphological variants in twins have only been minimally explored in the literature to date (Bailey et al., 1995; Voracek & Dressler, 2007). Since the twin design is a powerful tool in research to explore the genetic and environmental contributions to phenomena of interest, previous researchers such as Links et al. (1980) and Compton et al. (2011) have called for more studies in twins in order to better understand the heritability of morphological variants such as MPAs. Links et al. specifically recommended studies of MZ twins discordant for MPAs to explore potential environmental contributions to the development of morphological variants.

1.9.6.2 Measures/Assessment

Despite the popularity of the Waldrop scale for measuring MPAs, it presents major limitations including the small number of items assessed, low sensitivity, and low interrater reliability (Cheung et al., 2011). Furthermore, the scale was developed based on MPAs in a specific genetic disorder (i.e., Down syndrome). A study by Sivkov and Akabaliev (2003) examined the use of the scale in TD subjects and found poor internal consistency in the scale and recommended the creation or use of more reliable scales in the study of morphological variants. As noted earlier, the aforementioned Autism Dysmorphology Measure (Miles et al., 2008) has been used in only a few other studies to date and has only been validated to assess dysmorphology in children with ASD rather than other types of NDDs like ADHD, which may overlap with ASD.

The assessment of morphological variants overall is highly subjective. Even exams performed by highly trained and experienced individuals are subject to examiner bias. Automated facial analysis systems (e.g., F2G, 3dMD, etc.) now exist to measure some morphological variants objectively, thereby limiting examiner bias. Research is currently underway or has already been conducted (Aldridge et al., 2011; Gurovich et al., 2017; Obafemi-Ajayi et al., 2015; Pantel et al., 2018; Tripi et al., 2019) to explore the ability of these technologies to conduct morphological assessments, though further studies using this technology to assess morphological variants, especially in comparison to in-person clinical assessments, are needed.

1.9.6.3 Methods

Ozgen and colleagues (2011) specifically addressed limitations in studies to date on MPAs in children with ASD in particular and noted the following issues: “a. lack of standardization of the nomenclature and the absence of uniform diagnostic criteria; b. patients with different ethnic backgrounds were included; c. patients were not physically examined by the investigators specifically for the study; d. lack of control data or use of biased populations; e. relatively small sample sizes; f. no reports on interrater reliability; g. lack of consideration of gender effect” (p. 24). Even though these issues were identified almost a decade ago, research in the area of morphological variants only appears to be starting to address some of these limitations. For example, the introduction of HPO terminology in the past decade has helped standardize the language used to describe morphological variants. Additionally, the use of automated systems may help decrease some of the challenges and bias that may exist when humans examine patients with

different ethnic or racial backgrounds, as was found to be the case in a recent study with F2G (Lumaka et al., 2017).

2 AIMS

The aims of this doctoral thesis are to examine morphological variants associated with NDDs using a unique cohort of twins with detailed clinical and automated morphological assessments, neuroimaging, and molecular genetics (1) to identify characteristics of morphological variants that can support NDD screening and risk assessment, (2) to test whether it is possible to obtain reliable morphological assessments using low-cost automated technology, and (3) to utilize the twin design to explore the potential genetic and environmental influences on the development of morphological variants. The hypotheses for the various studies are that (1) an excess of clinically and automatically assessed morphological variants will be present individuals with NDDs compared with TD controls, (2) there will be a high convergence of clinical and automated morphological assessment, and (3) the presence of morphological variants will be highly correlated in MZ compared with DZ twins.

2.1 STUDY I– MINOR PHYSICAL ANOMALIES IN NEURODEVELOPMENTAL DISORDERS: A TWIN STUDY

The aims of Study I were 1) to explore the type and number of MPAs in twin pairs from RATSS who were concordant or discordant for NDD phenotypes and TD twin controls and 2) to examine the relationship between morphological variants in twins pairs by zygosity.

2.2 STUDY II– 2D:4D RATIO IN NEURODEVELOPMENTAL DISORDERS: A TWIN STUDY

The aim of Study II was to explore differences in the 2D:4D ratio in twin pairs from RATSS who were concordant or discordant for NDD phenotypes and TD twin controls.

2.3 STUDY III– NEURODEVELOPMENTAL AND MORPHOLOGICAL PHENOTYPES OF CARRIERS WITH 12q12 DUPLICATIONS

The aim of Study III was to describe the neurodevelopmental, behavioral, and morphological phenotype of a twin pair from RATSS who were found to have an inherited duplication on chromosome 12, along with phenotypes from participants found through an international database and the literature who had a similar duplication.

2.4 STUDY IV– CLINICAL VERSUS AUTOMATED ASSESSMENTS OF MORPHOLOGICAL VARIANTS IN TWINS WITH AND WITHOUT NEURODEVELOPMENTAL DISORDERS

The aims of study IV were to 1) determine agreement between clinical assessment of morphological variants (which were assessed in Study 1) and automated assessment of morphological variants (i.e., using F2G); 2) report on the use of automated assessment of facial images of twin pairs recruited through RATSS to examine the type and number of morphological variants present in participants in relationship to the presence or absence of NDD diagnoses; and 3) determine if faces of those with NDDs are distinguishable from those with TD.

2.5 SIGNIFICANCE OF STUDIES

The significance of these studies relates to both clinical work and to society as a whole. The use of assessments for morphological variants, including automated assessments, to identify children who may be at risk for NDDs, has the potential to be a valuable and cost-effective tool for clinical providers to help support diagnosis of NDDs. These assessments may also allow subgrouping of individuals with various NDDs based on the presence of morphological variants, which could be valuable for future treatment or interventions. Although previous studies have looked at the use of provider-completed measures to determine the presence of morphological variants, assessments using automated technologies (such as F2G) could help improve accuracy and may reduce the time and costs associated with in-person, clinical assessments.

3 METHODS

3.1 ETHICAL CONSIDERATIONS

3.1.1 Permits and Consent

Prior to the start of the RATSS project, ethical approval was received from the national Swedish and regional ethics board in Stockholm (dnr: 2016/1452-31 and dnr: Ö32-210). Before participation in the RATSS, participants and their parents/legal guardians (if applicable) provided consent for their involvement in the study. Since children were involved in the study, the assent of the children and the consent of the parents or legal guardians were obtained prior to participation. As some participants had cognitive impairments, it was essential to determine through interviews, medical records, clinical judgment, and discussion with legal guardians (when applicable) whether or not these participants were able to provide informed consent. This was done to ensure participants were aware of their involvement in the study and their rights as a research study participant (e.g., ability to decline study procedures, withdraw participation in the study, right to obtain information stored on them, etc.). The consent form for the study was approved by the ethical review board and was sent to the participants and their parents or legal guardians in advance, as well as reviewed again at the start of the in-person visit for the study. Participants and parents or legal guardians were offered a chance to ask questions and could select which parts of the study they provided assent/consent for participation. No participants were required to complete any part of the study for which they did not want to be involved.

3.2 RISKS AND BENEFITS

Researchers designed the RATSS study to maximize benefits and minimize risk to participants and their families. A research nurse accompanied the participants and their parents or legal guardians through most of the data collection procedures and was available for questions before, during, and after the in-person visit to the research center. Participants were offered compensation for the burden of participation in the study in a form of a gift card commensurate with the time and effort required of the participants and their parents or legal guardians to take part in the study. Participants were also provided reimbursement for transportation to the study site, loss of income or any other related expenses, and were offered accommodation at a nearby hotel if this was needed to participate in the study. The study procedures were performed in such a way to minimize fatigue on the participants and their families. With the various biological samples collected on participants in the RATSS

project (i.e., saliva, blood, MRI scans, etc.), participants were informed of any abnormalities identified from the samples (e.g., pathological or clinically significant variants) and were referred for any previously undetected problems. Prior to participation in some elements of the study (e.g., neuroimaging), safety questionnaires were completed by the participants to determine eligibility for participating in the particular element of the study. Feedback on the results of behavioral testing was also provided to the participants, if requested.

Since personnel in the RATSS collected data and biological samples from participants, confidentiality was critical and was maintained throughout the study by all research staff involved in RATSS. Personal data was handled in accordance with Swedish law. Each participant was assigned a unique identification code for the project. Biological samples were handled and stored according to Swedish law. The studies in this thesis specifically examined physical features in participants, both through in-person physical examinations and through medical photography. The use of photography in particular to identify individuals at risk for neurodevelopmental disorders was recently discussed in an article on the ethics of facial phenotyping by Kong (2019). Kong specifically discusses issues that arise when researchers use photographs to identify features in participants with NDDs that may further stigmatize individuals or even lead to issues resulting in eugenics. These concerns brought forth by Kong (2019) are extremely important to consider in studies such as the ones included in the thesis, which all examine physical or facial features in participants. To address these issues, all participants in RATSS were informed of the purpose for conducting physical examinations, as well as medical photographs of their bodies, along with their rights as research participants to participate in components of the RATSS that included physical examinations, as well as medical photography.

RATSS recruits a sample of twin pairs, particularly MZ twins discordant for ASD and ADHD, along with DZ twins and TD control twins, in order to collect a variety of behavioral data and biological samples from the twins with the overall aim to "understand the complexity of genotype-environment-phenotype interactions in ASD and ADHD" (Bölte et al., 2014, p. 164). In accordance with that aim, RATSS intends to benefit participants with NDDs broadly through "the identification of environmentally mediated biomarkers, the emergence of candidates for drug development, translational modeling, and new leads for prevention of incapacitating outcomes" (Bölte et al., 2014, p. 165). Participants were recruited from the entirety of Sweden and targeted recruitment was done

to ensure the sample had an adequate number of participants with suspected or diagnosed NDDs and did not disproportionately exclude any groups that were necessary to achieve the overall study aim.

3.3 STUDY DESIGN AND PARTICIPANTS

3.3.1 Roots of Autism and ADHD Twin Study in Sweden

The studies in this thesis were based on the matched case-control or the co-twin control design within the RATSS (Bölte et al., 2014).

3.3.1.1 Subsamples

Participants in the studies in this thesis from RATSS were recruited from 2011 to 2017 through a variety of methods. The primary source of recruitment for the twin pairs is through the Child and Adolescent Twin Study in Sweden (i.e., CATSS, Anckarsäter et al., 2011). Additional methods of recruitment include advertisements in journals for national interest organizations, referrals from clinical units (e.g., child psychiatry, habilitation centers, pediatric clinics), and the Swedish patient registry (Bölte et al., 2014). Initial recruitment of participants in the project focused on children as young as eight years of age and adolescents, but has expanded in recent years to include young adults. As of June 2019, 207 twin pairs and 2 trios of triplets (representing 220 MZ individuals, 186 DZ individuals, and 14 individuals with pending zygosity) have participated in the RATSS. Table 1 outlines the subsamples from RATSS for each of the studies in this doctoral thesis, including the number of participants in each study, the number of MZ versus DZ twins, percentage of males to females, percentage of twins with diagnoses of NDDs versus those with TD, and the mean, standard deviation, and range of ages of participants. Study III involves a rare, inherited genetic duplication discovered in a twin pair from RATSS and an additional five participants that were not part of RATSS that are included in the case series that were identified from DECIPHER, a genetic variant database, and through a previously published study (Wang et al., 2010). The information related to these five participants is not included in Table 1, rather only in the manuscript.

Table 1. Subsamples from RATSS by study.

Study	# of Participants	# of MZ Twin Pairs	# of DZ Twin Pairs	% M:F	% with ASD	% with ADHD	% with any NDD	% with TD	Age Range (M, SD)
I	116	51	7	57:43	24	32	53	47	9-23 years (14.0, 3.4)
II	238	70	49	55:45	19	27	44.5	55.5	8-29 years (16.2, 5.2)
III	2	1	N/A	2 Males	100	100	100	0	17 years (NA)
IV	290*	81	59	56:44	25	28	46.5	53.5	8-31 years (16.2, 5.3)

#=Number; %= Percentage; MZ= Monozygotic; DZ= Dizygotic; M:F=Male:Female Ratio; ASD=Autism Spectrum Disorder; ADHD=Attention-Deficit/Hyperactivity Disorder; NDD=Neurodevelopmental Disorder; TD=Typical Development; M=Mean; SD=Standard Deviation
*Includes 1 pair with pending zygosity

3.3.1.2 Procedures/Data Collection

Data collection in the RATSS took place generally over the course of three days in Stockholm, Sweden. The participants were assessed and samples were collected by trained and licensed behavioral and medical professionals. Behavioral data and biological samples collected in RATSS are illustrated in Figure 3, with those of particular importance to this doctoral project highlighted in bold and described in further detail below, including morphological assessments, behavioral assessments, neuroimaging, and saliva.

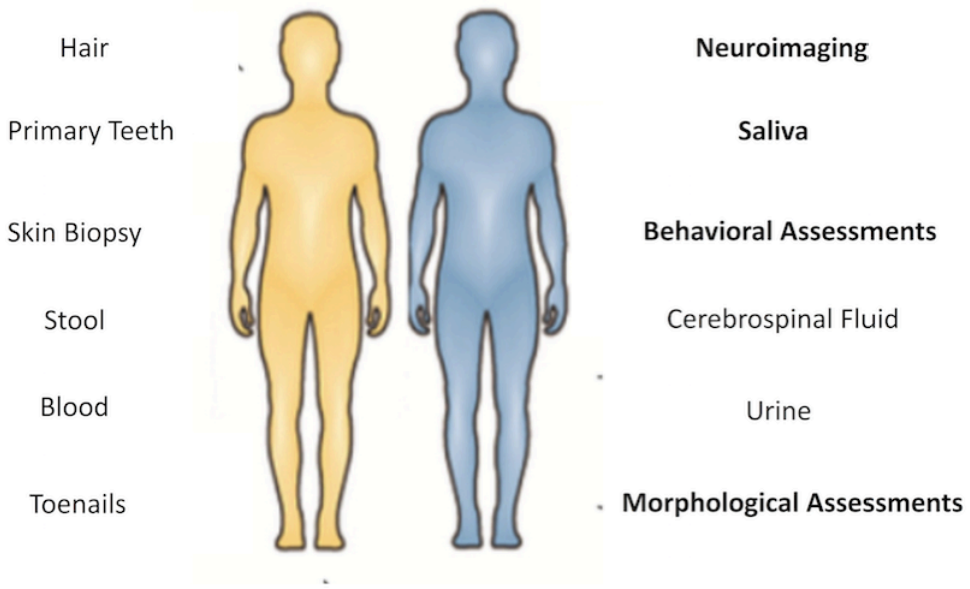

Figure 3. Illustration of biological and behavioral samples collected from twin pairs in RATSS.

3.3.1.2.1 Morphological assessments

Morphological assessments in the RATSS included three general elements: 1) in-person clinical assessments; 2) medical photography of participants' hands and measurement of

finger digits; and 3) medical photography of the participants' faces that were then scanned for facial morphological variants (FMVs) using F2G.

For participants recruited in RATSS between 2011 and 2013, two experienced, clinical geneticists conducted complete or nearly complete in-person clinical assessments of 116 participants. The clinical geneticists together developed a comprehensive head-to-toe checklist of 217 morphological variants, with an additional 8 variants for a genital exam in males only. The checklist was originally developed in Swedish and based on the *London Dysmorphology Database, Elements of Morphology*, and long-standing clinical expertise. The checklist was later translated into English by this PhD candidate, who is a pediatric nurse fluent in English, with the assistance of a pediatric psychiatrist, research nurse, and geneticist who were all fluent in Swedish and English. The translated checklist in English was then reviewed by the two geneticists who originally developed it to ensure the proper Swedish-to-English translation of the terms used to describe the morphological variants. The checklist was then further translated into HPO terms, and the two geneticists again reviewed and approved the translation into HPO terms where appropriate (see Appendix 1 for original and translated checklist). The checklist was used to guide and document the in-person clinical assessments, which took about 1 hour to complete for each twin pair. The assessments were primarily jointly completed by both geneticists and any disagreements between the geneticists on findings were resolved on the spot. The geneticists marked either "yes" or "no" on the checklist regarding the presence of each variant in the individual participants. In general, the presence a morphological variant resulted in a score of "1" for each variant identified on the participant to create a total score of the number of morphological variants present in each individual.

Medical photos (2D) were taken of both the right and left palmar and dorsal surfaces of hands of participants in RATSS at the Karolinska Hospital Medical Photography Lab. Using the palmar surface photographs of participants' hands, the 2nd and 4th finger digit (see Figure 4) were measured on both the right and left hands of participants by two raters. The measurements were taken from the midpoint of the arc defining the tip of both the 2nd and 4th finger digit vertically to the most proximal crease of each finger digit in the palm of the hand with a program called ImageJ (Schindelin, Rueden, Hiner, & Eliceiri, 2015). The average of these measurements between Raters 1 and 2 was used to calculate the 2D:4D ratio. The raters were blinded to the participants' diagnoses of NDDs versus TD. Both raters assessed the usability of the hand photographs to determine if there were

measurements for the 2nd or 4th finger digit on either hand that were unusable (e.g., digit not flat on table surface for photograph, hands or digits curled or cupped). If a participant lacked measurements by either rater for the 2nd or 4th finger digit on either the right or left hand due to the issues noted above, the participant (and their co-twin) were removed from the final analysis due to the inability to calculate an accurate 2D:4D ratio (i.e., 19 total twin pairs were removed).

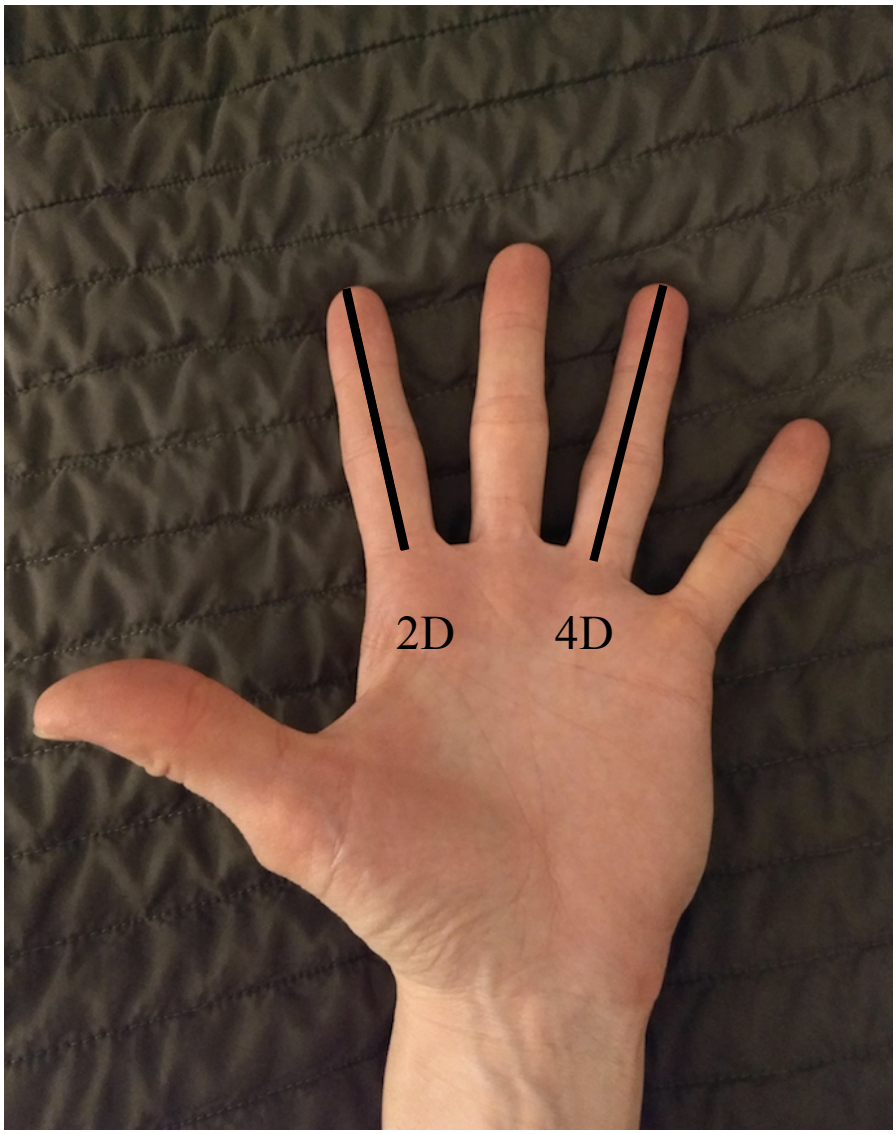

Figure 4. 2nd and 4th finger digit measurements.

Facial photos (2D) were also taken of participants in RATSS in the Karolinska Hospital Medical Photography Lab. The facial photos were securely transferred to F2G and then analyzed anonymously through the DeepGestalt system for the presence of any FMVs based on HPO terminology. The DeepGestalt system first detects the face in each photograph and then 130 facial points which are used to take the measurements. The measurements are then fed into a deep convolutional neural network which results in a list

of syndrome and gene matches (Basel-Vanagaite et al., 2016; Gurovich et al., 2017; Lumaka et al., 2017). From there, the system is able to provide a list of phenotypic features using HPO terms that are present in the individual based on the syndrome and gene matches. The features were reported back to the research team by F2G in a spreadsheet format.

3.3.1.2.2 Behavioral assessments

Participants in RATSS received a variety of assessments to help determine the presence of NDDs and other psychiatric conditions. The assessments primarily took place in-person during the study visit or were completed via questionnaires by the participants prior to or during the visit. The assessments were conducted by trained clinicians. Through a consensus process, the participants received diagnoses of NDDs or TD. Additionally, concordancy related to diagnoses for the twin pairs was determined during this process. For example, if one twin was diagnosed with ASD and their co-twin was diagnosed with ADHD, then the pair was considered ASD Discordant, ADHD Discordant, but NDD concordant. The various behavioral assessments conducted that are of interest to this doctoral thesis are included in Table 2, along with their reference and purpose overall in the RATSS.

Table 2. Description of instruments from RATSS used in studies.

Instrument	Reference	Purpose of Instrument in RATSS
Adult Behavior Checklist (ASEBA)	Achenbach, T.M., & Rescorla, L.A. (2003). *Manual for the ASEBA Adult Forms & Profiles*. Burlington, VT: University of Vermont Research Center for Children, Youth, & Families.	General assessment of behavior and functioning in adults, includes scales on "Attention Problems" and "Total Problems"
Autism Diagnostic Observation Schedule (ADOS-2)	Lord, C., Rutter, M., DiLavore, P., Risi, S., Gotham, K., & Bishop, S. (2012). *Autism Diagnostic Observation Schedule-2nd Edition (ADOS-2).* Los Angeles, CA: Western Psychological Services.	Diagnosis of ASD
Autism Diagnostic Interview-Revised (ADI-R)	Rutter, M., Le Couteur, A., & Lord, C. (2003). *The Autism Diagnostic Interview-Revised (ADI-R).* Los Angeles, CA: Western Psychological Services.	Diagnosis of ASD
Child Behavior Checklist (ASEBA)	Achenbach, T.M., & Rescorla, L.A. (2000). *Manual for the ASEBA School-Age Forms & Profiles.* Burlington, VT: University of Vermont Research Center for Children, Youth, & Families.	General assessment of behavior and functioning in children, includes scales on "Attention Problems" and "Total Problems"
Diagnostic Interview for ADHD in Adults (DIVA 2.0)	Kooij, J.J.S. (2010). *Diagnostic Interview for ADHD in Adults 2.0 (DIVA 2.0).* Amsterdam: Pearson	Diagnosis of ADHD in adults

Instrument	Reference	Purpose of Instrument in RATSS
Kiddie Schedule for Affective Disorders and Schizophrenia (K-SADS)	Kaufman, J., Birmaher, B., Brent, D., Rao, U., Flynn, C., Moreci, P.,…Ryan, N. (1997). Schedule for Affective Disorders and Schizophrenia for School-Age Children-Present and Lifetime Version (K-SADS-PL): Initial reliability and validity data. *Journal of the American Academy of Child & Adolescent Psychiatry. 36*(7), 980-988. doi:10.1097/00004583-199707000-00021	Diagnosis of ADHD in youth
Leiter International Performance Scale- Revised	Roid, G.H. & Miller, L.J. (1997). *Leiter International Performance Scale-Revised: Examiner's Manual.* Wood Dale, IL: Stoelting.	Non-verbal intellectual abilities; diagnosis of ID
Peabody Picture Vocabulary Test, Fourth Edition (PPVT-4)	Dunn, L.M. & Dunn, D.M. (2007). *Peabody Picture Vocabulary Test, 4th Edition.* San Antonio, TX: Pearson.	Verbal intellectual abilities; diagnosis of ID
Social Responsiveness Scale-2 (SRS-2)	Constantino, J.N. (2005). *Social Responsiveness Scale (SRS).* Los Angeles, CA: Western Psychological Services.	Measurement of autistic traits
Wechsler Adult Intelligence Scale-IV (WAIS-IV)	Wechsler, D. (2003). *WAIS-IV Wechsler Intelligence Scale for Adults 4th Edition: Technical and Interpretive Manual.* San Antonio, TX: Pearson.	IQ in adults; diagnosis of ID
Wechsler Intelligence Scale for Children-IV (WISC-IV)	Wechsler, D. (2003). *WISC-IV Wechsler Intelligence Scale for Children 4th Edition Technical and Interpretive Manual*. San Antonio, TX: Pearson.	IQ in children; diagnosis of ID

3.3.1.2.3 Neuroimaging

Neuroimaging, in the form of MRI, was performed on participants in RATSS using a 3 Tesla MR750 GE scanner at the Karolinska Institutet MR Center. Both T1 and T2 images, along with functional MRI (fMRI) and diffusion tensor imaging (DTI) were obtained through an approximately 50-minute scanning session on each participant who consented to the procedure and was eligible based on their medical history. The images obtained from the MRI were initially reviewed by clinical radiologists at the Karolinska Hospital for the presence of neuroradiological variants, including either incidental findings (IF) or pathological findings that required further clinical follow-up. Due to the number of radiologists reading the images over the 8-year span of the RATSS so far (2011-2019), an experienced, independent, pediatric neuroradiologist was recruited to re-read the T1 and T2 images in a blinded fashion to conduct a standardized and consistent assessment of all participants' MRI images for the presence of neuroradiological variants.

3.3.1.2.4 Saliva

Saliva is obtained from both participants and their biological parents (if available) during RATSS to use for both zygosity and genetic testing. Details related to the extraction of DNA from these samples can be found in Stamouli et al. (2018). Genotyping was performed on the DNA using the Infinium PsychArray-24 v1.1 (Illumina Inc., San Diego, California, USA). The PLINK/1.07 software (Purcell et al., 2007) was used to confirm zygosity from genotype data by estimating the identify of descent after quality control was performed on the data and any single nucleotide variants with a minor allele frequency less than 0.05 were removed (Stamouli et al., 2018). For a few twin pairs, zygosity was determined either through a short tandem repeat kit (Promega Powerplex 21) or through a zygosity questionnaire used in CATSS (Willfors, Carlsson, et al., 2017).

3.3.1.3 Statistical Methods

Statistics utilized in this doctoral project included descriptive statistics, interrater agreement, receiver operating characteristic (ROC) curves, correlational statistics, tests of difference, and generalized estimating equations (GEE). Table 3 provides an overview of the methods used by study. SPSS version 24, R version 3.3.2, and R Studio version 1.0.44 were used to perform the statistics. Further detail related to statistical methods can be found in the individual papers in this thesis.

Table 3. Overview of statistical methods by study.

Statistical Method	Study I	Study II	Study III	Study IV
Descriptive statistics	X	X	X	X
Interrater agreement	X	X		X
ROC curves				X
Correlational statistics	X (Spearman)	X (Spearman)		
GEE	X	X		X
Tests of difference	X (Mann-Whitney U)			X (GEE)

3.3.1.3.1 Descriptive statistics

Descriptive statistics in this thesis were performed to describe the number, percentage, measures of central tendency [i.e., mean (M) and median (Md)], standard deviation (SD), and ranges of values.

3.3.1.3.2 Interrater agreement

Measures of interrater agreement were used to compare assessments between clinical geneticists conducting in-person exams on participants and a physician trained in dysmorphology (Study I) or with the F2G findings (Study IV). Statistics related to agreement were calculated using the *irr* package (version 0.84) in R Studio.

3.3.1.3.3 ROC curves

ROC curves were calculated through the F2G Research Platform to examine if participants with NDDs could be differentiated from those with TD using facial features detected with the F2G system. ROC curves are useful in that they look at the amount of separation between two entities (Hajian-Tilaki, 2013). In the case of facial analysis of participants with NDDs compared with TD, the curve plots the true-positive rate (sensitivity) of being able to discriminate participants on facial features on the y-axis against the false-positive rate to create an area under the curve (AUC) (Hajian-Tilaki, 2013). AUC values nearing 1.0 indicate increasing accuracy of the system in being able to discriminate among individuals with varying diagnoses, while values closer to 0.5 indicate a more random ability of the system to discriminate among individuals (Tape, n.d.).

3.3.1.3.4 Correlational statistics

Spearman correlations were used to assess the relationship between zygosity and 1) number of MPAs in twin pairs (Study I) and 2) 2D:4D finger digit ratios (Study II) due to the non-normal distribution of the number of MPAs and finger digit ratios, respectively, in participants in the studies.

3.3.1.3.5 GEE

Since the RATSS involves data based on twins, standard correlational measures are not entirely appropriate due to the paired nature of the data and the fact that twins share some factors like environment and genetics and are, therefore, not independent (Carlin, Gurrin, Sterne, Morley, & Dwyer, 2005). The statistic used to calculate associations, as well as differences between variables, was twin modelling with GEE. The model examines associations and differences between-pairs and within-pairs. The between-pairs model operates like a standard linear or logistic regression, except that it accounts for the shared factors within twin pairs using clustered standard errors, while the within-pairs model specifically accounts for factors shared such as environment and genetics. In the within-pairs model, Carlin et al. (2005) state that stronger beta values in MZ versus DZ twins indicate the potential role of the environment, while weaker beta values for MZ compared

with DZ pairs may indicate a stronger role of genetics as the MZ twin pairs are almost perfectly matched on genetic factors. Both beta values and odd ratios, including corresponding standard errors, 95% confidence intervals, and p-values, can be calculated using the GEE model. The *drgee* package (version 1.1.6) in R Studio (Zetterqvist, Vansteelandt, Pawitan, & Sjolander, 2016) was used to perform the GEE statistics in this thesis.

3.3.1.3.6 Tests of difference

The Mann-Whitney U test was performed to assess the significance of the difference in number MPAs that were identical and different in MZ versus DZ twins (Study I). The GEE model was used to compare the difference in the percentage of specific FMVs in those with NDDs to those with TD (Study IV).

3.3.1.4 Variables explored by study

The variables explored in this thesis through statistical analysis that were obtained through the biological and behavioral samples in the RATSS are listed in Table 4 below by study number. Further details related to these variables and how they were analyzed in each study can be found in the individual papers in this thesis.

Table 4. Variables explored by study.

Variables	Study I	Study II	Study III	Study IV
NDD diagnosis	X	X	X	X
Demographics	X	X	X	X
IQ	X	X	X	X
SRS	X	X		X
ASEBA				X
Count of physical anomalies (MPAs, FMVs, etc.)	X			X
2D:4D finger digit measurements/ratio		X		
MRI findings	X			

4 RESULTS

4.1 STUDY I

4.1.1 MPAs in ASD and other NDDs

Descriptively, Study I found individuals with NDDs had higher median numbers of MPAs (Md=range of 4 to 9 MPAs by NDD diagnosis) compared with those with TD (Md=3 MPAs). Statistically, however, higher MPAs were only significantly associated with a diagnosis of ASD (crude odds ratio =1.29, p=.047). Twin pairs concordant for ASD had the highest median number of MPAs (Md=9 MPAs). Due to the association of MPAs with ASD, autistic traits, as measured through the SRS-2, were also correlated with the number of MPAs and found to be significant in the entire sample [ß=3.02, Standard Error (SE)=.98, 95% confidence interval=1.09 – 4.94, p=.002], indicating that every MPA present in an individual corresponded to an approximately 3-point increase in their SRS-2 score, indicating the presence of higher autistic traits.

4.1.2 MPAs and IQ

IQ was negatively associated with the presence of MPAs (ß=-.95, SE=.32, 95% confidence interval=-1.59 – -.32, p=.003), so that for every MPA present in an individual there was an approximately 1-point decrease in their IQ, indicating a trend for lower IQs in individuals with increasing MPAs.

4.1.3 MPAs in MZ twins

The number of MPAs were highly correlated in MZ (r_s= .88, p<.001), but not DZ (r_s= -.19, p<.676) twin pairs. MZ twin pairs had both the higher number of identical MPAs (Md=4) compared with DZ twins (Md=1; z=-2.764, p=.006) and a smaller median difference in specific MPAs present (Md difference=2) compared with DZ twins (Md difference= 4; z=-1.066, p=.287).

4.2 STUDY II

4.2.1 2D:4D Ratio Relationships with Diagnoses of NDDs or ADHD

Study II found only weak relationships between the diagnosis of NDDs and a lower 2D:4D finger digit ratio in both males (between-pairs model, ß=-.014, 95% confidence interval=-.025 – -.002, p=.019) and females (within-pairs model, ß=-.017, 95% confidence interval=-.035 – .000, p=.050), indicating a decrease in the ratio by either .014 for males or .017 for

females. Additionally, a weak relationship was found between a diagnosis of ADHD and a lower ratio in males (between-pairs model, ß=-.015, 95% confidence interval=-.027 – -.003, p=.012).

4.2.2 2D:4D Ratio in Males Compared with Females

Overall, males had a lower median 2D:4D finger digit ratio (Md for overall hand=.992, interquartile range=.970 and 1.015) in comparison with females (Md for overall hand=1.010, interquartile range=.981 and 1.031). When examining the ratio in the various NDDs by sex, males had lower Md ratios for each disorder compared with females.

4.3 STUDY III

4.3.1 Rare Genetic Duplication with Incomplete Penetrance in Twins and Others with MPAs and NDDs

A genetic duplication affecting chromosome 12 long arm (12q12) was found in a twin pair in RATSS. Five other individuals with a similar size and location of duplication were identified through the literature or DECIPHER, a database including individuals with genetic abnormalities. Individuals with the duplication had learning difficulties, cognitive impairment, language and gross motor delays, and at least one NDD (i.e., intellectual disability, ADHD, ASD). Individuals with the duplication were primarily males with morphological variants present in head shape, forehead, eyes, vision, ears, nose, oral-facial region, and toe digits.

4.4 STUDY IV

4.4.1 Agreement between Clinical and Automated Assessment of Morphological Variants

When comparing in-person clinical assessments of morphological variants to variants identified through F2G, agreement was high to complete (78.3 – 100%) for 36 FMVs mutually assessed by both raters, though this level of agreement was primarily based on the agreement between raters with the non-findings of FMVs.

4.4.2 Facial Morphological Variants in Individuals with NDDs

The number of FMVs was not associated with a diagnosis of a NDD in either the between-pairs or within-pairs model. However, there was a weak association between IQ and the number of FMVs (ß=-1.538, 95% confidence interval=-2.961 – -.115, p=.0341), indicating that every one facial FMV present corresponded to a 1.5-point decrease in IQ. Facial

features of individuals with ASD, ADHD, and a diagnosis of any NDD were not significantly distinguishable from those with TD (AUC range .561-.584, $p>.05$).

5 DISCUSSION

5.1.1 Discussion on Study Results

The studies in this thesis found higher numbers of morphological variants, including MPAs and FMVs, along with lower 2D:4D finger digit ratios, in individuals with NDDs compared to those with TD. However, the statistical relationship between increasing morphological variants in individuals with diagnoses of NDDs was not always significant. Notable exceptions were the increasing numbers of MPAs in individuals with ASD and autistic traits found in Study I, similar to results from previous studies and a meta-analysis finding increased amounts of MPAs in individuals with ASD (Ozgen et al., 2013; Ozgen et al., 2010). When looking at the relationship of morphological variants and IQ, studies I and IV found an association between increasing morphological variants and decreasing IQ, similar to an older study in participants with morphological variants and ASD by Links et al. (1980) where they also found lower IQs with the presence of morphological variants.

Study I also points to the genetic basis for MPAs in particular, with MZ compared with DZ twins having both similar amounts, as well as types, of MPAs, even if the MZ twins did not share NDD diagnoses. The use of the twin design in the studies helped to determine the genetic nature of morphological variants, which has been called for previously in the literature by Links et al. (1980) and Compton et al. (2011), but was never before performed in a sample of participants with NDDs as an overarching diagnostic category.

Lower finger digit ratios have been found previously in studies in individuals with NDDs, primarily ASD (Al-Zaid et al., 2015; de Bruin et al., 2009; Honekopp, 2012b; Manning et al., 2001), but the results in Study II did not find a significant relationship between the lower ratio and a diagnosis of ASD, similar to the findings in a recent large study of individuals with ASD versus TD by Guyatt et al. (2015) (Guyatt et al., 2015). However, weak relationships existed between lower ratios in individuals with diagnoses of any NDDs and in males with ADHD.

Study III suggested specific morphological variants present in individuals with a duplication on Chromosome 12, which mirrored some of the same body areas affected by individuals previously identified with a deletion syndrome in that region (Adam, Mehta,

Knight, Hall, & Rossi, 2010; Carlsen, Frengen, Fannemel, & Misceo, 2015; Failla et al., 2008; Weng, Luo, & Hou, 2018).

When the examination of morphological variants was limited to the face only, as in Study VI, no association between the number of FMVs and a diagnosis of ASD, ADHD, or any NDD was present. Although previous studies have identified distinctive facial phenotypes in boys with ASD (Aldridge et al., 2011; Hammond et al., 2008; Obafemi-Ajayi et al., 2015), as well as the possibility of more masculine and/or less feminine facial features in individuals with ASD (Bejerot et al., 2018; Tan et al., 2017), limiting the clinical assessment of an individual for morphological variants to the facial region alone failed to distinguish participants with NDDs from those with TD in study IV. Angkustsiri et al. (2011) compared in-person ratings of MPAs in photographs of faces and hands of individuals with ASD, developmental delay, and TD and also pointed out the limitations of assessments relying on just the face and hands in identifying morphological variants. However, their study found significantly higher rates of MPAs in individuals with ASD and developmental delay compared with those with TD.

The overall results of the studies in this thesis find increased morphological variants descriptively in individuals with NDDs, but do not point to any distinct variants statistically that can be used in early diagnosis of NDDs. This supports the conclusion by Ozgen et al. (2010) that the number of morphological variants, rather than specific variants, may relate to NDD diagnoses. Using the total number of morphological variants versus identification of specific morphological variants in an individual may result in identifying someone with dysmorphism as defined by the work of Miles et al. (2005) and could be the more useful outcome of morphological assessments. The results of this thesis also point to the potential for the assessment of morphological variants to serve as a possible marker for individuals undergoing diagnosis of NDDs who may benefit from further assessment. Further assessment could include genetic testing to identify genetic variants as potential etiological mechanisms or eventually to use to subgroup individuals with NDDs that may benefit from certain types of treatments or interventions. However, further research is needed to examine the relationship between morphological variants and subgroups.

5.1.2 Strengths and Limitations of Studies

5.1.2.1 Strengths

The RATSS project for which the studies in this thesis are based includes many strengths, namely, that the RATSS explores multiple facets related to the development of NDDs, including the role of genetics and environment, biological process, and subsequent behavioral outcomes. The sample recruited in RATSS is based not only on a clinical sample, but also a population-based sample, thereby limiting selection bias and allowing for some ability to make statements for the broader population. The study involves deep phenotyping of participants through the use of standardized behavioral measures, biological specimen collection, and in-person assessments like physical examinations and MRI scans. The sample is almost equally balanced to include males and females, which has been a critique of previous research on NDDs using higher numbers of male participants based on the skewed sex ratio, especially in the area of ASD (Lai, Lombardo, Auyeung, Chakrabarti, & Baron-Cohen, 2015). Additionally, the sample includes dimensional, along with categorical, measures of NDD symptoms. Additional strengths pertaining directly to the methods used in the studies in this thesis are the use of multiple raters to determine interrater agreement in Studies I, II, and IV, the use of clinical geneticists who were experts in dysmorphology for in-person clinical assessments in Studies I, III, and IV, and the use of novel automated technology to assess morphological variants in Study IV in order to limit some of the subjective nature of assessments completed in-person.

5.1.2.2 Limitations

Limitations to the RATSS include that the sample in the study is from one country (i.e., Sweden) and was limited to recruitment of higher functioning participants with NDDs who could participate in the various procedures. This may have prevented some participants who may have been too severely impaired by their diagnoses to participate. Another limitation is examiner bias during in-person morphological assessments by clinical geneticists due to the inability to blind these examiners to the participants' diagnoses as they met participants face-to-face. Limitations also exist in the checklist used to assess the morphological variants during the in-person clinical morphological assessments as it was not previously validated. The study also lacks parent photos or morphological assessments in parents to assess for familial tendency of morphological variants. The large number of radiologists who read the MRI scans of participants in the study over the years is a limitation because they may not have approached the scans in a consistent manner. Even though the RATSS sample includes many participants with NDDs, smaller sample sizes existed in the NDD

subgroups, which may have resulted in the inability to identify significant associations between diagnoses and variables of interest in the studies in this thesis. Finally, one could question the ability generalize the findings from the RATSS to the greater population, especially non-twins. It is important to note that the results of the studies in this thesis were consistent with the results of other cited studies conducted on singletons, thereby suggesting that being a twin may not place an individual at increased risk for either morphological variants or NDDs.

5.1.3 Clinical Implications of Studies

Clinical assessments for the presence of morphological variants may be able to aid in the identification of individuals who would benefit most from additional testing such as genetic testing when undergoing diagnosis of a NDD, especially if resources for additional testing are limited in a region or country. Recommendations exist within the US to conduct genetic testing on any child who receives a diagnosis of a NDD such as ASD and ID (Johnson et al., 2007; Moeschler et al., 2014; Schaefer et al., 2013; Volkmar et al., 2014), yet similar recommendations have not yet been made worldwide. Despite best practice recommendations for genetic testing, studies from the US show parents report around 35% of children with ASD actually receive genetic testing and less than 20% of parents report receiving information about genetic testing from their health care provider (Kiely, Vettam, & Adesman, 2016; Li et al., 2016). Genetic testing in the diagnosis of NDDs offers many benefits, including providing individuals with information about potential genetic causes, the ability of the information to guide some treatment decisions, providing families with information about recurrence risks of the NDD, as well as the possibility for early intervention related to detection of genetic conditions for which an individual may be at risk (Tammimies, Falck-Ytter, & Bölte, 2016; Tremblay et al., 2019; Vorstman et al., 2017). Numerous constraints have been presented regarding universal genetic testing for children diagnosed with ASD (Tammimies et al., 2016; Tremblay et al., 2019; Vorstman et al., 2017), including parental reactions to testing (e.g., blame, guilt, anxiety) when inherited genetic variants are identified, potential to find conditions or risk for conditions related to poor prognosis or serious disease, lack of training of health care providers related to genetic testing and interpretation of test results, parent thoughts that the genetic testing will not lead to changes in the treatment decisions, and the cost and availability of testing worldwide.

Although providing universal genetic testing offers benefits in terms of being able to expand the knowledge worldwide related to genetic variation in NDDs (Vorstman et al.,

2017), a first step to encourage more genetic testing may be to include the use of a the clinical assessment for morphological variants to help determine the presence and number of MPAs or morphological variants that could then serve as a screening tool for individuals who may be at greater risk for an underlying genetic cause for the disorder and therefore, may most benefit from genetic testing. In fact, Tammimies et al. (2015) showed 37.5% of children classified as having "complex ASD" based on the presence of six or more MPAs, a structural brain abnormality, and/or major congenital anomalies were found to have a positive genetic finding through both CMA and WES genetic testing. Previous studies also point to higher MPA scores as suggestive of potential genetic reasons for the etiology of ASD that may be due to sporadic or non-familial reasons, including de novo genetic changes (Miles et al., 2005; Tammimies et al., 2015). It has been suggested recently that targeted screening of children believed to be at high-risk for ASD (e.g., presence of first-degree relative with ASD, specific genetic conditions, etc.) may be a more efficient and cost-effective strategy than universal screening when it comes to early detection of NDDs (Yuen, Carter, Szatmari, & Ungar, 2018); thereby supporting the use of evaluations such as clinical assessments for morphological variants to identify children who may most benefit from early screening for NDDs.

In summary, the clinical assessment may help classify children with NDDs for whom additional testing, such as further genetic or diagnostic testing, should be prioritized in order to better understand potential etiology for the disorders, as well as potential co-morbidities or other factors that need to be considered in the evaluation and intervention related to the child undergoing NDD diagnosis.

6 CONCLUSION

6.1 FUTURE DIRECTIONS

Multiple avenues of research could be explored related to the outcomes so far in the studies in this thesis, as well as additional data collected through the RATSS project that could be analyzed in relationship to morphological variants. As previously noted, morphological variants, especially in the facial region, may mirror altered brain development due to simultaneous development in utero between the face and brain, which is guided by molecular signals (Jones, 2013; Marcucio et al., 2015). Comparing morphological variants (assessed either through clinical or automated assessments) with other measures utilized to evaluate children with NDDs, such as MRIs and genetic testing, may provide insight into the potential relationship between morphological variants, brain development, and genetics and would be a logical next study. Clinical or automated assessment of morphological variants may help identify children who could benefit from neuroimaging by exploring the relationship between morphological variants and the presence of abnormal MRIs. Currently, neuroimaging is not routinely recommended in the assessment of children for NDDs, like ASD (Filipek et al., 2000). Therefore, use of the clinical assessment may help prioritize children who would benefit most from neuroimaging in the diagnosis of NDDs. Additionally, a future study could explore the relationship between genetic variants identified through the genetic testing performed in RATSS (like presence and size of CNVs) with the presence of morphological variants, in line with previous studies which found increased morphological variants in individuals with genetic issues (e.g., Miles et al., 2005; Tammimies et al., 2015).

The RATSS collects prenatal and perinatal data from mothers of the twins. Since previous studies (Firestone & Prabhu, 1983; Links et al., 1980) have identified a relationship between increased MPAs and prenatal and perinatal complications, the effects of complications prenatally and perinatally in participants in RATSS could be explored in relation to the presence of morphological variants, especially those that differ within MZ pairs, as recommended by Links (1980).

Since the in-person clinical assessment still remains the standard for identification of morphological variants, further studies exploring the use newer morphological measures like the Autism Dysmorphology Measure (Miles et al., 2008) in samples with not only

ASD, but also other NDDs which often overlap with ASD like ADHD and ID, may be warranted to see if the measure performs well in other samples from those in which it had been initially studied. Additionally, it would be important to explore the use of the Autism Dysmorphology Measure by a variety of variety of clinicians (e.g., nurses), and not just physicians, to determine if other providers can accurately use the measure to assess morphological variants. If the measure can be used by multiple types of providers, this would help increase the availability of these morphological assessments for individuals undergoing diagnosis of NDDs. Additionally, the area of physical examinations in NDDs could use the development of some standard guidelines worldwide so that any healthcare provider seeing an individual suspected of having a NDD would have a consistent and evidence-based approach to the examination so that all individuals would receive the recommended assessments, screenings, and associated next steps in a similar manner.

Many of the participants in RATSS are recruited from CATSS, and in CATSS, a measure called the Dysmorphic Concern Questionnaire (DCQ) (Oosthuizen, Lambert, & Castle, 1998) is administered to participants at 18 years of age. The self-administered questionnaire consists of seven questions that assess for concerns an individual may have related to dysmorphic appearance. In CATSS, an additional question is included related to the whether a disease or some type of damage to one's appearance has caused an individual to worry about their appearance. The original study regarding the development and validation of the DCQ questionnaire was conducted on 90 patients with varying psychiatric disorders admitted to a psychiatric ward, including over half with schizophrenia and nearly 40% with affective disorders (Oosthuizen et al., 1998). The study did not find a relationship between increasing scores on the DCQ (indicating greater dysmorphic concerns) and ratings of dysmorphology using the Waldrop scale. However, it is important to note that this sample was small and included adults already admitted to a psychiatric ward and not a population-based sample. Therefore, a future possible study could match the same participants in RATSS with those in CATSS who have turned 18 years to compare in-person clinical or automated assessments of morphological variants in RATSS to self-rated dysmorphology in CATSS using the DCQ.

6.2 CONCLUSION

The studies in this thesis aim to identify characteristics of morphological variants that can support NDD screening and risk assessment, to test whether it is possible to obtain reliable morphological assessments using low-cost automated technology, and to utilize the twin

design to explore the potential genetic and environmental influences on the development of morphological variants. The studies in this thesis found no distinct morphological variants to distinguish those with NDDs from those with TD, but increasing numbers of morphological variants may be a marker to identify individuals who would benefit from further assessments, such as genetic testing, which is not currently routinely recommended in the diagnosis of individuals with NDDs. Although automated methods of assessment for morphological variants demonstrated high to complete agreement with in-person clinical assessments, the technology in this area is still emerging and is currently limited to only particular body regions (e.g., face). Consequently, this technology may miss morphological variants present in other areas of the body that may help determine appropriate next steps for an individual suspected of having an NDD. Further research is needed on the use of these automated technologies to identify morphological variants, as well as the expansion of these technologies to assess morphological variants in the entire habitus. Finally, as expected, MPAs were found to be highly correlated in MZ compared with DZ twins, indicating a strong genetic basis for MPAs.

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Appendix 1. Morphological variants assessed in-person and translated from Swedish to English and then into HPO terms and aligned with items assessed with Face2Gene.

Checklist (Swedish)	Checklist (English)	HPO Feature Term and ID	Face2Gene Item
Panna	**Forehead**		
Panna hög	High forehead	High forehead (348)	
Panna låg	Low forehead	Low hairline (294)	
Panna buktande	Bulging forehead	Prominent Forehead (11220)	
Panna sluttande	Sloping forehead	Sloping forehead (340)	
Panna bred	Broad forehead	Broad forehead (337)	
Panna smal	Narrow forehead	Narrow forehead (341)	Narrow forehead
Tinningar insjunkna	Sunken temples		Narrow forehead
Hår eller hårfäste avvikande	**Hair or Hairline**		
Hårfäste högt i nacke och panna	High anterior and posterior hairline	High posterior hairline (12891) and High anterior hairline (9890)	High anterior hairline
Hårfäste lågt i nacke och panna	Low anterior and posterior hairline	Low anterior hairline (294) Low posterior hairline (2162)	
Hårfäste treflikigt	Three-peak hairline	Pointed frontal hairline (4544)	
Cows lick	Cowlick		
Widows peak	Widow's Peak	Widow's peak (349)	
Hår tjockt	Thick hair	Thick hair (100874)	
Hår tunt	Thin hair	Fine hair (2213) or Sparse scalp hair (2209)	Sparse scalp hair
Hår strävt	Rough hair	Coarse hair (2280)	
Hår ljust	Bright hair	Fair hair (2286)	
Hår mörkt	Dark hair		
Hår lockigt	Curly hair	Curly hair (2210)	
Hår rakt	Straight hair		
Hår pigmentförändringar	Pigment anomalies in hair	Abnormality of hair pigmentation (9887)	
Hirsutism	Hirsutism	Hirsutism (1007)	

Checklist (Swedish)	Checklist (English)	HPO Feature Term and ID	Face2Gene Item
Ögonbryn/fransar avvikande	**Eyebrow or Eyelashes**		
Ögonfransar långa	Long eyelashes	Long eyelashes (527)	
Ögonfransar korta	Short eyelashes	Short eyelashes (10764)	
Ögonfransar saknas	Absent eyelashes	Sparse or absent eyelashes (200102)	
Ögonfransar dubbla	Double eyelashes	Multiple rows of eyelashes (8496)	
Ögonbryn högt placerade	High placed eyebrow	Abnormal location of eyebrow (40296)	
Ögonbryn lågt placerade	Low placed eyebrow	Abnormal location of eyebrow (40296)	
Ögonbryn raka	Straight or horizontal eyebrow	Horizontal eyebrow (11228)	
Ögonbryn bågformade	Highly arched eyebrow	Highly arched eyebrow (2553)	Highly arched eyebrow
Ögonbryn outvecklade lateralt	Underdeveloped eyebrow- laterally	Sparse lateral eyebrow (5338) or sparse eyebrow (45075)	Sparse eyebrow
Ögonbryn outvecklade medialt	Underdeveloped eyebrow- medially	Sparse medial eyebrow (25325) or sparse eyebrow (45075), Medial flaring of the eyebrow (10747)	Sparse eyebrow, Medial flaring of the eyebrow
Ögonbryn saknas	Missing eyebrow	Absent Eyebrow (2223)	
Synofrys	Synophrys	Synophrys (664)	Synophrys
Hud avvikande	**Skin**		
Hud tjock	Thick skin	Thickened skin (1072)	
Hud tunn	Thin skin	Thin skin (963)	
Hud ljus	Light skin	Hypopigmentation of the skin (1010)	
Hud mörk	Dark skin	Hyperpigmentation of the skin (953)	
Hud torr	Dry skin	Dry skin (958)	
Hud fet	Oily skin		
Hud sammetslen	Velvety skin	Soft skin (977)	
Hud åldrad	Aged skin	Excessive wrinkled skin (7392)	
Hud överskott	Excess skin	Redundant skin (1582)	
Hud stram	Tight skin	Stiff skin (30053)	
Hud onormal fettdistribution	Abnormal fat distribution		
Café au lait-fläckar	Café au lait spots	Cafe au lait spots (957)	
Hypopigmenteringar	Hypopigmentation	Hypopigmented skin patches (1053)	
Nevi	Nevi	Hyperpigmented nevi (7481)	

Checklist (Swedish)	Checklist (English)	HPO Feature Term and ID	Face2Gene Item
Hemangiom	Hemangioma	Hemangioma (1028)	
Tumörer	Tumor	Neoplasm of the skin (8069)	
Blåsor	Blisters	Skin vesicle (200037)	
Papler	Papules	Skin-colored papule (25512)	
Ansiktform avvikande	**Facial Form**		
Ansiktsform runt	Round face	Round face (311)	
Ansiktsform fyrkantigt	Square face	Square face (321)	
Ansiktsform triangelformat	Triangular face	Triangular face (325)	Triangular face
Nacke avvikande	**Neck**		
Nacke bred	Broad neck	Broad neck (475)	
Nacke kort	Short neck	Short neck (470)	
Nacke ökat nackskinn	Redundant nuchal skin	Redundant neck skin (5989)	
Nacke pterygium	Neck webbing	Webbed neck (465)	
Ögon avvikande	**Eyes**		
Synavvikelse	Visual Impairment	Abnormality of vision (504)	
Ögonen små	Microphtalmia	Micophtalmia (568)	
Ögonglob saknas	Missing eyeballs or Anophthalmia	Anophthalmia (528)	
Iris avvikelser	Iris abnormalities	Aplasia/Hypoplasia of the iris (8053), Abnormality of the iris (525) Abnormal iris pigmentation (8034), Iris hypopigmentation (7730), Asymmetry of iris pigmentation (200064)	
Ögonen prominenta	Prominent eyes	Large eyes (1090) or Propotosis (520)	Propotosis
Ögonen djupt liggande	Deeply set eyes	Deeply set eye (490)	
Hypertelorism	Hypertelorism	Hypertelorism (316)	Hypertelorism
Hypotelorism	Hypotelorism	Hypotelorism (601)	Hypotelorism
Ögonspringor korta	Short palpebral fissure	Short palpebral fissure (12745), Blepharophimosis (581)	Blepharophimosis
Ögonspringor långa	Long palpebral fissure	Long palpebral fissure (637)	

Checklist (Swedish)	Checklist (English)	HPO Feature Term and ID	Face2Gene Item
Ögonspringor nedåtsluttande	Downslanted palpebral fissure	Downslanted palpebral fissure (494)	Downslanted palpebral fissures
Ögonspringor uppåtsluttande	Upslanted palpebral fissure	Upslanted palpebral fissure (582)	Upslanted palpebral fissure
Epikantus	Epicanthus	Epicanthus (286)	Epicanthus
Telekantus	Telecanthus (506)	Telecanthus	Telecanthus
Ptos	Ptosis	Ptosis (508)	Ptosis
Kolobom	Coloboma	Coloboma (589)	
Telangiektasier	Telangiectasias	Conjunctival telangiectasia (524)	
Avvikande tårproduktion	Abnormal tear production		
Ögonbottenundersökning avvikande	Abnormality in retina	Abnormal retinal morphology (479)	
Linsdislokation	Lens dislocation		
Näthinneavlossning	Retinal detachment	Retinal detachment (541)	
Glaukom	Glaucoma	Glaucoma (501)	
Katarakt	Cataract	Cataract (518)	
Munregion avvikande	**Mouth Region**		
Mun stor	Wide mouth	Wide mouth (154)	
Mun liten	Narrow mouth	Narrow mouth (160)	
Överläppen tältformad	Tented upper lip	Tented upper lip vermillion (10804), Tented philtrum (11825)	Tented upper lip vermillion, Tented philtrum
Läpparna tjocka	Thick lip	Thick lower lip vermilion (179), Thick upper lip vermilion (215)	Thick upper lip vermilion, Thick lower lip vermilion
Läpparna smala	Thin lip	Thin upper lip vermilion (419) Thin lower lip vermilion (10282)	Thin upper lip vermilion
Läppspalt	Cleft lip	Cleft lip (410030)	
Andra avvikelser, t.ex. gropar, upphöjningar eller avvikelser av frenulum	Other anomalies- e.g., dimples, ridges, or deviations of frenulum		
Gom hög	High palate	High palate (218)	
Gom spetsig	Angled palate	High palate (218)	
Uvula bred	Broad uvula	Broad uvula (10809)	

Checklist (Swedish)	Checklist (English)	HPO Feature Term and ID	Face2Gene Item
Uvula bifid	Bifid or cleft uvula	Bifid uvula (193)	
Gingiva tjock	Thick gingiva	Gingival overgrowth (212)	
Tunga stor	Large tongue	Protruding tongue (10808)	
Tunga grov	Furrowed tongue	Furrowed tongue (221)	
Tunga missbildad	Malformed tongue		
Mikrognati	Micrognathia	Micrognathia (347), Retrognathia (278), Abnormality of the chin (306)	Abnormality of the chin
Prognati	Prognathia	Mandibular prognathia (303), Abnormality of the chin (306)	Abnormality of the chin
Filtrum avvikande	**Philtrum**		
Filtrum långt	Long philtrum	Long philtrum (343)	Long philtrum
Filtrum kort	Short philtrum	Short philtrum (322)	Short philtrum
Filtrum utslätat	Smooth philtrum	Smooth philtrum (319)	Smooth philtrum
Filtrum djupt	Deep philtrum	Deep philtrum (2002)	Deep philtrum
Tänder avvikande	**Teeth**		
Bett trångt	Dental crowding	Dental crowding (678)	
Bett brett	Widely spaced teeth	Widely spaced teeth (687)	
Tänder glesa	Oligodontia	Oligodontia (677)	
Tänder stora	Macrodontia	Macrodontia (1572)	
Tänder små	Microdontia	Microdontia (691)	
Avvikande tandform	Abnormal tooth shape	Abnormality of dental morphology (6482)	
Emaljdefekter	Enamel defect	Abnormality of dental enamel (682)	
Hypodonti	Hypodontia	Hypodontia (668)	
Adonti	Anodontia	Anodontia (674)	
Extra tänder	Additional teeth	Abnormal number of teeth (6483)	
Onormal tid för tanderuption	Delayed or advanced eruption	Delayed eruption of teeth (684) or advanced eruption of teeth (6288)	
Andra tandavvikelser	Other dental abnormalities		

Checklist (Swedish)	Checklist (English)	HPO Feature Term and ID	Face2Gene Item
Öron avvikande	**Ears**		
Små	Microtia	Microtia (8851)	
Stora	Long ear	Long ear (400004)	
Dysplastiska	Dysplastic ears	Abnormality of outer ear (356)	
Lågt sittande	Low-set ear	Low-set ears (369)	Low-set ears
Bakåtroterade	Posteriorly rotated ear	Posteriorly rotated ear (358)	
Helices nervikta	Overfolded helix	Overfolded helix (396)	Overfolded helix
Helices tjocka	Thick helices	Prominent ear helix (9004)	
Helices tunna	Thin helices	Thin ear helix (9005)	
Örsnibben avvikande form	Abnormal earlobe form	Abnormality of earlobe	
Preaurikulära bihang, gropar/fåror	Preauricular tags or pits	Preauricular pit (4467), Periauricular skin pits (100277), Preauricular skin tag (384)	
Näsa avvikande	**Nose**		
Näsa liten	Small nose	Slender Nose (417)	
Näsa stor	Prominent Nose	Prominent nose (448)	
Näsa lång	Long nose	Long nose (3189)	
Näsa kort	Short nose	Short nose (3196)	Short nose
Näsa platt	Flat nose	Depressed nasal tip (437)	
Näsa nedåtböjd	Hooked nose	Nose, hooked (9000066)	
Näsa uppåtböjd	Upturned tip of the nose	Abnormality of the nasal tip (436)	Abnormality of the nasal tip
Näsrygg hög	High nasal bridge	Prominent nasal bridge (426)	Prominent nasal bridge
Näsrygg låg	Low nasal bridge	Short nasal bridge (3194)	
Näsrygg bred	Broad nasal bridge	Wide nasal bridge (431)	Wide nasal bridge
Näsrygg smal	Thin nasal bridge	Narrow nasal bridge (446)	
Nästipp bred	Broad nasal tip	Broad nasal tip (455)	Broad nasal tip
Nästipp smal	Narrow nasal tip	Narrow nasal tip (11832)	
Näsown kort	Short columella	Short columella (2000)	
Nässkiljevägg slutar den nedanför näsvingen	Low hanging columella	Low hanging columella (9765)	

Checklist (Swedish)	Checklist (English)	HPO Feature Term and ID	Face2Gene Item
Näsvingar små	Narrow naris	Narrow naris (9933), Underdeveloped nasal alae (430)	Underdeveloped nasal alae
Näsvingar anteverterade	Anteverted nares	Anteverted nares (463)	Anteverted nares